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The Effects of Intermittent Hand Cooling on Internal Body Temperature, Exercise Performance, and Inflammatory Cytokines During Hyperthermic Exercise

Robert A. Huggins
robert.huggins@uconn.edu

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Robert Arthur Huggins, PhD

University of Connecticut, 2014

Limited research exists regarding peripheral hand cooling (HC) modalities with negative pressure during American football and it is unclear if a reduction in internal body temperature will positively influence body temperature, perceptual measures, athletic performance, and circulating inflammatory cytokines. Two separate research studies, an environmental controlled laboratory and observational field study, examined HC while wearing a full American football uniform. Study#1: Rectal temperature was significantly decreased from minute 66 through post-exercise in the HC with fluid condition ($p<0.05$). Substantial elevations in cortisol, testosterone, and creatine kinase (range 3% to 48%) and inflammatory markers (IL-8 and IL-6) were different over time ($p=0.001$, $p=0.001$), respectively. •IL-8 responses were increased for the HC alone group compared to the HC with fluid group ($p=0.008$) indicating increased pro-inflammation. The performance battery increased 5-8% during HC with fluid replacement compared to HC alone and control conditions with balance being most positively influenced (21% to 37%). Reduced thermal strain likely allowed for greater effort during performance tasks. Study#2: Gastrointestinal temperature T_{GI} was not significantly different between the HC and control conditions at either research site on any day. Moderate and varied effects were observed for other physiological, perceptual, and performance data. Environmental conditions were mild relative to historical average conditions and may have influenced the efficacy of the HC device. In conclusion, HC coupled with hydration reduced physiological strain, inflammation and increased performance during laboratory testing, but not during field testing. Future research in various contexts is required to enhance our understanding of this cooling modality.

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Robert Arthur Huggins

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The Effects of Intermittent Hand Cooling on Internal Body Temperature, Exercise Performance, and Inflammatory Cytokines During Hyperthermic Exercise

Presented by

Robert Arthur Huggins, B.S., M.Ed

Major Advisor: ____

Douglas J. Casa, Ph.D.

Associate Advisor: ____

Lawrence E. Armstrong, Ph.D.

Associate Advisor: ____

Jeffrey Anderson, M.D.

Associate Advisor: ____

Carl M. Maresh, Ph.D.

Associate Advisor:____

Elaine C. Lee, Ph.D.

University of Connecticut
2014

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TABLE OF CONTENTS

CHAPTER 1: REVIEW OF THE LITERATURE

Thermoregulation, Heat Balance and Uncompensable Heat Stress

The ability to compensate or to maintain one's internal body temperature during exercise in the heat has been shown to be critical for performance.[1–4] When the amount of heat gain equals the amount of heat loss the body is said to be in a compensable heat stress (CHS) situation.[5] When the heat gain exceeds the amount of heat loss the body is said to be in an uncompensable state. Uncompensable heat stress (UCHS) is defined as an environment where the heat stress index or HSI is equal to 1.0 (HSI = evaporation required divided by the evaporation max OR Ereq/Emax). When the HSI is equal to 1.0, there is a resultant net storage of heat in the body and as a result the body is unable to thermoregulate appropriately.[5] A great deal of research currently exists examining thermoregulation during CHS[1,4,6,7] situations as well as the factors that improve one's removal of heat from the body. The influence of heat acclimation during CHS[2,8–10] have been the primary focus of researchers in an effort to optimize performance in the heat and minimize the risk for heat illness. Research in UCHS is less prominent and has focused primarily on the tolerance of individuals while wearing clothing that reduces the ability of sweat to evaporate.[2,8,9,11–15] A great deal of the UCHS research was spearheaded by the U.S. and Canadian military forces[12,13,15] in an attempt to determine safe limits for soldiers while wearing chemical protective clothing. Protective clothing increases the challenge of thermoregulation because water vapor permeability is limited and the rate of heat exchange is reduced.[5] Other types of clothing during UCHS that have been researched related to civilian activities include firefighter gear[16] and football equipment.[17–20] These types of clothing, although just as dangerous and often less regulated, have not been examined as extensively as chemical protective clothing. However it is important to note that the principles behind UCHS mechanism remain the same. Many of the findings of chemical protective research can be applied to civilian situations.

Evaporative Heat Loss:

Evaporative heat loss is the body's most efficient mechanism of heat loss and occurs when a there is a temperature gradient created between the skin and the surrounding environment.[21–23] The larger the gradient, the greater the vaporization of sweat. With each droplet of sweat, heat is removed from the body (approximately 2400kJ/1L of sweat) resulting in a reduced body temperature. Environmental factors such as the ambient temperature as well as the relative humidity greatly influence the ability of the sweat to evaporate.[24–26] For example, if the relative humidity is high the amount of water in the air is increased. This increase in water content in the surrounding environment makes it difficult to utilize the sweating mechanism as efficiently as possible, because the sweat is unable to evaporate. In addition to the surrounding environment, clothing also a key factor influencing the evaporative mechanism.[8,13,27,28] During exercise in the heat, clothing and its insulation capacity or clo value greatly influence the body's evaporative capacity.[27] The greater the surface area of the body that is covered, the less the evaporative heat dissipation. Clothing creates a microclimate between the clothing and the skin surface which directly influences the ability of sweat to vaporize.[27] The sweat that is not evaporated from the surface of the skin will not result in heat transfer and the body will not be able to dissipate heat. Many clothing scenarios in the work force, military as well as sport put the body at a disadvantage, which often results in an uncompensable heat stress situation.

Heat Balance:

During uncompensable heat stress the evaporative heat loss required to maintain a steady state exceeds the maximal evaporative capacity of the environment and the body stores heat. In order to fully understand heat loss versus heat gain an understanding of the heat balance equation is essential.

Heat Balance Equation: $S=M-W\pm(E\pm R\pm C\pm K)$

- S= A positive S value represents the gain in heat storage by the body where a negative value represents net heat loss.
- M= the metabolic heat production and is determined by indirect calorimetry.
- W= the external work performed by the person
- E = the evaporative exchange which is divided into wet and dry
    - Dry is dependent on the temperature gradient
    - Wet loss is the evaporation of water or sweat.
- R= the radiant heat exchange
- C= the conductive exchange
- K= the convective exchange

UCHS Research:

During UCHS the environment, intensity, and the clothing are just a few of the factors influencing heat balance making it extremely difficult to regulate body temperature and reach a plateau.[15] The ability to reach a plateau is commonly seen in compensable heat stress situations, but during UCHS the internal body temperature and heart rate continue to rise until the individual becomes exhausted or collapses. Research has focused on the ability of the human body to tolerate UCHS, however factors that are commonly beneficial in CHS are not completely shared by UCHS situations. Research in the area of heat acclimation and short-term aerobic training have been shown to have limited improvement in exercise heat tolerance time of soldiers[8,12] during exercise in the heat. However research examining the long-term aerobic fitness has been shown to provide some degree of protection.[29,30] Two of the proposed mechanisms for increased tolerance in chronic aerobically trained athletes are that they have an increased ability to tolerate higher temperatures and advantageous cardiovascular adaptations help to reduce the cardiovascular strain associated with exercise in the heat. Furthermore hydration status has proven to be very influential in mitigating the rise in internal body temperature and increasing tolerance time.[9,13] Tolerance time in UCHS is often described as the time it takes for the body to reach an upper limit for body temperature and heart rate or the inability to continue exercise. Many research studies conducted in controlled laboratory situations look at the tolerance time during UCHS situations.[8,9,12–15,29–31] Often due to human ethical limitations researchers cease exercise when internal body temperature reaches 39.5-40.0˚C or heart rate exceeds their age predicted max for a specific duration of time. A more comprehensive summary of UCHS research on humans is presented in Table 1.1 below.

Table 1.1 Summary of Articles Examining Uncompensable Heat Stress

Article	Number of Subjects	Subject Demographics	Fitness level HA status	Study Type	Type of Exercise	Equipment	Type of Temp	Environmental Temperature	Temperature Results	Heart Rate Results	Key Points	Uncompensable or Compensable or Both
Armstrong (2010)	10 male football players	23.8 y; 183.9cm; 117.41kg; BF=12.59%	Healthy; unacclimatized	Lab	Repetitive Box lifting Treadmill walk 60 min	1. Partial Uniform 2. Full Uniform 3. Control	Tre	33°C (48-49%rh)	Full- 2.37 Tre increase; rate=.042 Partial-2.36 Tre increase; rate=0.034 Ctrl- 1.81 Tre increase; rate=.026	Full-180bpm; Partial 178bpm; Ctrl 164bpm	Full and Partial Uniform resulted in greater physiological strain. BF% influenced time to exhaustion. Lean BM influenced Trec.	Uncompensable and compensable
Aoyagi (1993)	16 males (7males intervention 9 males control)	27y, 1.76m, 82kg, $1.98m^2$42.1 VO2	8 weeks of endurance training followed by 6 days Heat acclimation	Lab	Treadmill walk 4.8km/h, 2%grade	1. Control (1.4clo) 2. Chem clothing (2.3clo)	Tre	40°C (30%rh)	HA Decreased rate of Tre increase by 0.1°C/h. HA decrased the resting Tre by 0.2°C. UT subjects were 0.1°C while ET were.2°C/hr	HA had no effect on HR in the ET group. Benefits of plasma vol expansion were reduced with chem clothing.	Rates of Tre increase were decreased after training. Heat acclimation improved Tre and Tsk when wearing NBC clothing. Tolerance times were unchanged.Endurnace training nor HA improved tolerance.	Uncompensable and compensable
Aoyagi (1994)	16 males		Heat acclimated for 6 days or 12 days at 40°C and 30%rh	Lab	Treadmill (1.34m/s, 0%) 150min 15 min active/15min rest	1. Control 2. Chem Clothing	Tre	40°C (30%rh)	Rate of increase was slower 0.1-0.2°C after HA.	HA reduced HR by 26bpm regardless of the days of HA or the clothing. Similar reduction of 8bpm with 6 and 12 days	HA of 12 days is no better than 12 days. Both 6 and 12 increased tolerance time by 15 min	Uncompensable and compensable
Aoyagi (1998)	Experiment 1: 16 males (8 per group)	G1: 25y;1.76m;83.1kg G2: 31y, 1.77m; 78.7kg	Military Personnel	Lab	Grp 1: 8 wks training followed by 6 days of HA; Pre and Post treadmill Test 1.34m/s 2% grade Grp 2: 8wks control followed by 6 days of HA; Pre and Post treadmill Test 1.34m/s 2% grade	1.Chemical Protective (2.4clo) 2. Control(1.4clo)	Tre	40°C (30%rh)	G1=Ctrl & Chem Tre:37.8°C, 37.7°C G2=Ctrl & Chem Tre:37.9°C, 37.6°C	G1=Ctrl and Chem HR:114, 134 G2=Ctrl and Chem HR:123, 134	No significant changes in tolerance times between groups. Wearing protective clothing, 8 week training did not improve rate of Tre rise but Tsk was higher after training. Grp 2 HA alone did not alter rate of Tre or Tsk rise. HA decreased overall values of Tre and Tsk. Endurance significantly decreased the HR after 30 min	Uncompensable and compensable
Aoyagi (1998)	Experiment 2: 16 males (8 per group)	Group 1:29y, 1.79m, 82.6kg, 48VO2 Group 2: 28y, 1.78m, 83kg, 47VO2	Military Personnel	Lab	G1:6 day HA G2: 12 day HAG1: Continuous exercise G2: Intermittent Exercise (15/15min)Pre and Post Treadmill Test 1.34m/s 0% grade	1. Chemical Protective 2.Control	Tre	40°C (30%rh)	Pre G1= (Ctrl, Chem) 38°C, 37.9°C Pre G2=37.9°C, 37.8°C Post G1=37.8°C, 37.7°C Post G2=37.5, 37.6	Pre HA: G1=(Ctrl,Chem)118,116 G2=126,118 Post HA:G1= 110, 108 G2=107, 110	HA reduced HR by 8-19bpm regardless of clothing or HA period of 6 or 12 days. Normal Clothing HR decrease following HA of 12 days was less.	Uncompensable and compensable
Cheung (1998)	8 males	29.3y,1.78m,75.6kg,56.5VO2,12.4%, $1.94m^2$	Military Personnel	Lab	Light Treamill 3.5km/hr; 0% or Heavy Treadmill 4.8km/h 4% grade in 3 different fluid conditions EU c Fluid,EU no fluid, hypo w fluid	1. Chemical Protective only	Tre	40°C (30%rh)	During light ex delta Tre was higher with H/F than Eu/F after 40 min. End mean temp was not different between hydration conditions. EUF, EUNF, HF Light were 38.9°C, 36.89°C,38.76°C. EUF, EUNF, HF Heavy were 38.69°C, 38.71°C, 38.74°C	Fluid replacement resulted in a significantly lower HR	Tolerance times were greater for the EU/F then for Eu/NF or EU/F trials. No differences in delta Tre were observed across conditions.	Uncompensable only
Cheung (1999)	16 males (8 moderate fitness and 8 high fitness)	18-40y, MF=30y;1.77m,84.7kg, 19.2%, $2.02m^2$ HF=27.9y, 1.77m, 76.8kg, 11.5%, $1.94m^2$	Moderate Fitness=<50VO2 max; High Fitness= >55VO2max	Lab	Treamill Exercise (3.5km/hr) Moderate fit group trained for 2 weeks in 22C for 60min per day	Chemical Protective Clothing(1.88clo)	Tre	40°C (33%rh)	Training (Delta Tre MF Pre=1.26°C, Post=1.09°C. End Tre MF Pre=38.38°C Post=38.2°1C); Heat Stress Test (End Tre MF Pre=38.7°C MF Post=38.61°C HF=39.15°C	MF Pre=146.9; MF Post=137.7bpm	The endpoint Tre and delta Tre and tolerance time remained higher in HF than either the MF pre or Post training. Short term aerobic trainin offers little benefit and does not substitute for high aerobic fitness. HF had a 20-25min longer tolerance time	Uncompensable only
Fox (1966)	5 male football player	16-18y;172-182cm;68-96kg:	Experienced High school football players	Lab	Treadmill 6mph 20min	1. Full uniform 2. Scrub suit	Tre	23-25°C (31-55%rh)	0.82°C Tre increase in Scrub suit;1.06°C Tre increase in Football gear	Full 196bpm; Scrub 181 MD=15bpm	Temp, HR and sweat loss were significantly increased in the equipment condition	Uncompensable and compensable

Article	Number of Subjects	Subject Demographics	Fitness level HA status	Study Type	Type of Exercise	Equipment	Type of Temp	Environmental Temperature	Temperature Results	Heart Rate Results	Key Points	Uncompensable or Compensable or Both
Hitchcock (2007)	5 college off-linemen	20y;194.6cm;132.7kg, BF 20%, SA 2.6m^2	Unacclimatized OF linement	Lab	Simulated drills (Drive block, treadmill run, bear crawl, wall sit, dot drill)30-81%VO2Max: avg 55%	1. Full Footbll Uniform 2.Helmet & Shoulder Pads 3.Helmet 4.Shorts	Tgi	28°C (55%rh)	Helmet+ShoulderPads was 0.3°C higher than helmet alone. No other significant differences	Heart rate was not significantly different	The full football equipment increases core temperature and energy cost. There was a significant increase in VO2max with helmet and shoulderpads compared to helmet alone and shoulder alone groups. Full did not differ. HR and VO2max were higher in this study than others	Uncompensable and compensable
Kraning (1991)	4 males	19-43y, 86kg, SA=1.92m^2	Moderately fit; healthy; Unacclimatized	Lab	1. Continuous Treadmill at 3.3 METs 2. Repeated 10 min exercise-rest patterns (4min walk, 2 min jog, 4 min rest) for 120 min	1. t-shirt,shorts, boots,wt belt 2. Work clothing and semipermiable chem protective gear	Tgi and Tes	30°C (6.3 Vapor Pressure)	Temp was 0.4°C higher at 60min	No significnat diff	During compensable (steady state was achieved) Uncomp= 33% greater rise with intermittent exercise. All subject completed the compensable condition. None of the uncompensable completed the protocol. Heat production for the walk, jog and rest were (201, 489, and 67 Watts/m^2	Uncompensable and compensable
Latzka (1998)	8 males	23y, 76kg, LBM 63kg, 56 VO2, 46.4L TBW	Heat acclimatized	Lab	Treadmill exercise 55% VO2 to exhuastion. Time ranged from 29.9-33.8min.	Chemical Protective Clothing in all cases 1.Control 2. Glycerol Hyperhydration 3. Water Hyperhydration	Tre	39°C (26dew pt)	Temp was similar in all (uncompensable situations 38.6-38.8°C). Change = 1.5-1.7°C	Heart rate was not significantly different (185-187bpm)	No difference between glycerol and water hyperhydration on TBW> Glycerol endurance time was 3.9min longer than ctrl but not water	All uncompensable
Mathews (1969)	9 males	74.5kg, SA 1.92m^2	Healthy;	Lab	Treadmill running (9.6km/hr an 30 min recovery)	1. Shorts 2. Football uniform 3. Shorts & backpack	Tre	25.6°C (33%Rh)	Temp increased 1.7, 1.1, and 1.4°C in uniform, shorts, and pack respecitvely	Heart rate at end of ex was 186, 168, 179 in uniform, shorts, and pack respectively	Evaporative cooling was 20 and 55kcal/m per hr less and sweating 128 and *3% more in uniform than shorts and pack respectively. BML was 2.97, 1.47, and 1.81 for the uniform, shorts, pack respectively.	Uncompensable and compensable
Mclellan (1999)	9 males	33.6y, 81.5kg, 1.97m^2,46.6VO2	Unacclimatized	Lab	Treadmill walking 3.5km/hr for 45min then seated rest for 15 followed by another 45 min walk	All uncompensable trials wearing NBC gear: 1. Morning; melatonin or placebo 2. Afternoon; melatonin or placebo	Tre	30°C (40%rh)	Tre final was 39.07, 38.97, 39.22, 39.08°C for the AM Mel, AM Plac, PM Mel and PM Plac respectively. Rate of heat storage was 103.9, 100.6, 100.6, 96.5W/m^2	Heart rate was not different between conditions and increased steadily over time	Melatonin had no impact on heat stress tolerance. Circadian rhythms influenced Tre with trials in early afternoon rising more.	All uncompensable
Montain (1994)	7 males	21y, 80.1kg, SA 2.0m^2, VO@max 52	Healthy soldiers; Heat acclimatized	Lab	Moderate Intensity and High Intensity Treadmill walking for 180min at 400 and 600W	1.Full (clo=1.5) 2.Partial protective clothing (clo=1.3)	Tre	Four trials in Two conditions: Desert and Tropical 1. 43°C, 20%rh) 2. 35°C 50%rh	**Fully Clothed:**ModInt Desert=38.6°C ModInt Trop=38.7°C, HighInt Desert=38.5°C, HighInt Trop 38.5°C **Partial:** 39.1, 38.8, 38.5, 38.5°C respectively	**Fully Clothed:** ModInt Desert=166bpm, ModInt Trop=160bpm, HighInt Desert=178bpm, HighInt Trop 173bpm **Partial:** MID 171, 167, 178, 173bpm	Wearing full clothing lowered the Tre point of exhaustion compared with partial. Heart rates were influenced by the exercise intensity clothing and climate. Tre at exhaustion was similar regardless of whether subjects were performing moderate of high intensity exercise in full clothing	Uncompensable and compensable
Sawka (2001)	12 males	27y, 80.4kg, 20%BF, SA 1.96m^2	Healthy male soliders; Heat Acclimatized	Field Study-Yuma, AZ	Moderate: 1.34m/s c 18kg; High: 1.56m/s 3.5 c 32.7lbs Mod Inten: Contin Moderate Int: Intermmit (30min, 10min rest) High Int: Cont HighInt:Intermittent Rest	MOPP IV Chemical Protective Clothing; (clo=2.11)	Tre	WBGT 29.4°C; wind (3.6m/s)	MC=38.6°C, MI=38.9°C, HC=39.0°C, HI 39.0°C	MC=165bpm, MI=171bpm, HC=179bpm, HI=172bpm	Core temp at exhaustion was not altered by intensity or exercise-rest cycles. Exhaustion from heat strin occurred in 33 or 48 trials 17/24 for Cont and 16/24 for intermitt. Exercise with rest cycles extend exposure time during MI but not Cont exercise. Exercise with rest reduced distance during High Int. Comparision to lab studies show the heat strain was greater in the field than lab	Uncompensable only
Schlader (2011)	8 males	34y;1.8m;70.1kg,SA 1.87m^2, 10.6%BF	Well trained cyclists	Lab	1. Self-paced 2. Fixed Intensity (70%VO2max) FI trial was performed 1st in all cases	Cycling attire	Tgi	40.6°C, 23%rh	FI Tgi=39.4°C and SP=39.1°C; FI rate of rise 0.108°C/min; SP=0.082°C/min	No differences in HR responses	When exercise is self paced, behavioral modification of metabolic heat production improves the compensability of the thermal environment. Self paced resulted in longer duration by 3 min than fixed intensity.	Uncompensable only
Selkirk (2001)	4 matched groups of (4males and 2 females) 24 total	21.2-25.2y;168.5-175cm,61.3-78.7kg, SA 1.69-1.93m^2	Trained High BF; Trained low BF; Untrained high BF; Untrained low BF	Lab	Treadmill walking 3.5km/h	protective clothing (1.88clo)	Tre	40°C, 30%rh	Tre Final for Tlow, Thigh, Utlow, Uthigh =39.48, 39.22, 38.58, 38.78°C respectively. Delta Tre=2.46, 2.12, 1.39, 1.52°C	At 60min: Tlow, Utlow,= 128.7 & 156.7bpm; Final HR not significant between gourps	Aerobic fitness, body fatness and heat storage each have an effect on tolerance. High Aerobic fitness = ability to tolerate higer Tre. Exercise time was greater in TLow 116min compared to Utlow 70 min ot THigh 82min indicating an advantage for both high aerbic fitness and low BF	Uncompensable only

There are many factors that influence UCHS tolerance in both a positive and negative manner. (see Table 1.2) Research has looked very carefully at these factors in an attempt to improve the tolerance time of athletes, laborers and military personnel during UCHS. Multiple research studies during UCHS have demonstrated that aerobic fitness,[29,30] body fatness,[29] and hydration[9,13,22] have been influential factors in the rate of rise of internal body temperature and tolerance time. Interestingly, heat acclimation in a compensable environment has been the focus for many athletes, warfighters and laborers, however heat acclimation using short-term aerobic training[14,32] has not been shown to aid in increasing tolerance during UCHS. This is opposed to the adaptations that we often see with individuals who are in compensable heat stress (CHS) situations. During CHS, heat acclimation[33,34] has been shown to increase the cardiovascular and thermal effects in a positive manner increasing exercise tolerance time and allowing the body to reach a state of thermal balance. This same method of heat acclimation prior to UCHS scenarios has been shown to have little influence on tolerance time. The increased sweat rate associated with heat acclimation caused dehydration to occur more quickly which can directly influence tolerance if fluids are not replaced.

Table 1.2

Factors That Increase UCHS Tolerance	Non-influential Factors	Factors That Decrease UCHS Tolerance
• Long term aerobic fitness • Low body fat% • Euhydration • Clothing with high evaporative capacity (low clo value) • Low intensity of exercise • Low ambient temp and relative humidity • Self pacing (increased tolerance time) • Intermittent exercise (increased time not peak temp) • Increased skin exposure to environment • Decreased metabolic rate	• Heat acclimation* • Increased sweat rate • Melatonin • Glycerol suppl.	• Low-Moderate Aerobic Fitness • High body fat% • Hypohydration of >2.5% • Clothing with low evaporative capacity (high clo value) • High ambient temp and %rh • Fixed pacing (reduced tolerance time) • Circadian Rhythm (increased afternoon temp) • Outdoor heat stress > indoor • Increased metabolic rate

*In full MOPP gear heat acclimation has no influence (Aoyagi) however heat acclimation has been shown to provide some positive cardiovascular changes

<u>UCHS and Football:</u>

The American Football uniform can quickly transform into UCHS depending on the type of uniform worn,[20,21] the environmental conditions,[24] level of heat acclimatization,[35] and most importantly the intensity of the exercise session. Further examination of the uniform ensemble by Armstrong et al.[20] demonstrated that great physiological strain was present when athletes were in full and partial football uniform and that percent body fat influenced the time to exhaustion and that lean body mass influenced rectal temperature. In separate studies, Fox et al.[17] and Mathews et al.[18] also demonstrated the negative effects of wearing football equipment and demonstrated that body temperature, heart rate and sweat loss were increased while wearing equipment[17] and furthermore that evaporative cooling was 20-55kcal/m^2 per hr less than shorts only.[18] Epidemiological data examining weather conditions[24] and heat illness rates in football[36,37] support the notion that environment is a major contributor specifically the level of humidity with August being the month of greatest risk. When factors such as equipment, environment, heat acclimatization and intensity are all taken into context we can gain a more thorough understanding of how UCHS can occur. Little has been done specifically quantifying the thermal stress associated with football in the field setting. Temperature variation responses have been examined by Coris et al[38] during two-a-day practices in lineman with higher temperatures being observed in the a.m. compared to p.m. This provided some of the seminal work describing the variation in body temperature and the cumulative heat stress that occurs over multi-day sessions. Godek et al.[39] similarly observed that when football players wore pads that body temperatures were significantly elevated and that lineman demonstrated higher body temperatures compared to backs.[40] Last, Yeargin et al.[35] examined heat-acclimatized high school football players and determined that they can safely complete the initial days of preseason football practice in moderate environmental conditions using well-designed practice guidelines.

To my knowledge, these four studies are the only research currently quantifying the level of heat stress during football. No field research has captured football players when all factors, equipment, environment, intensity, and lack of heat acclimatization are present. Future research is needed to examine football players during practices in the heat with one or more of these factors. Once, these factors have been more adequately quantified, strategies for prevention such as cooling before, during or after practice should be examined in depth to provide the most effective means to keep body temperature in a compensable state where performance is optimized.

Hydration and Body Temperature

Hydration increases the ability of the human body to thermoregulate by increasing total body water which aids in the maintenance of total blood volume.[41–43] Hydration increases one's blood volume and results in an increase in blood flow to the skin allowing the body to cool more effectively through the evaporation of sweat. When the body does not replace fluids during exercise, the body is unable to thermoregulate as efficiently and sweat rate declines as demonstrated in Figure 1.1 below by Sawka et al.[44] This decline is sweat production increases the amount of heat stored in the body making it difficult for one to thermoregulate.

Dehydration during exercise in the heat has been well documented during exercise in the heat.[44–49] Some of the earliest research has demonstrated that there is a strong link between hydration status and thermoregulation during exercise.[50,51] Fluid loss has been associated with increases in body temperature between 0.1 and 0.43°C for every additional 1% body mass loss[23,42–44,46,49,52–63] with the 1992 study by Montain and Coyle[43] depicting the effects of graded dehydration on hyperthermia in a classic manner as depicted below in Figure 1.2 below. Unpublished recent meta-analyses results examining 18 research studies [23,42–44,46,49,52–63] during hyperthermic exercise in the heat, further refined this increase to precisely 0.22℃ for every additional 1% body mass loss. (see Figure 1.3)

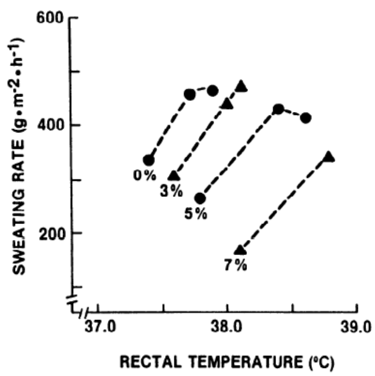


FIG. 4. Plot of group mean sweating rate and final exercise rectal temperature values for initial 3 exercise bouts (2 bouts for 7% hypohydration) during 4 experimental conditions.

Figure1.1

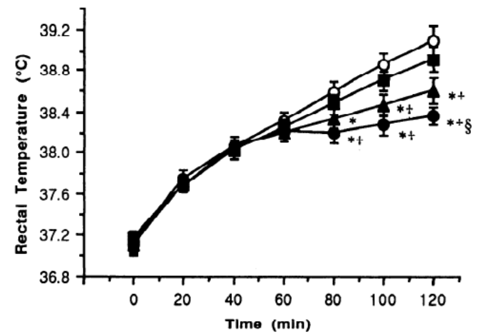


FIG. 1. Esophageal (n = 7) and rectal (n = 8) temperatures during 120 min of exercise when no fluid or small, moderate, or large volume of fluid was ingested. Values are means ± SE. *Significantly lower than no fluid, $P < 0.05$. †Significantly lower than small volume of fluid, $P < 0.05$. §Significantly lower than moderate volume of fluid, $P < 0.05$.

Figure 1.2

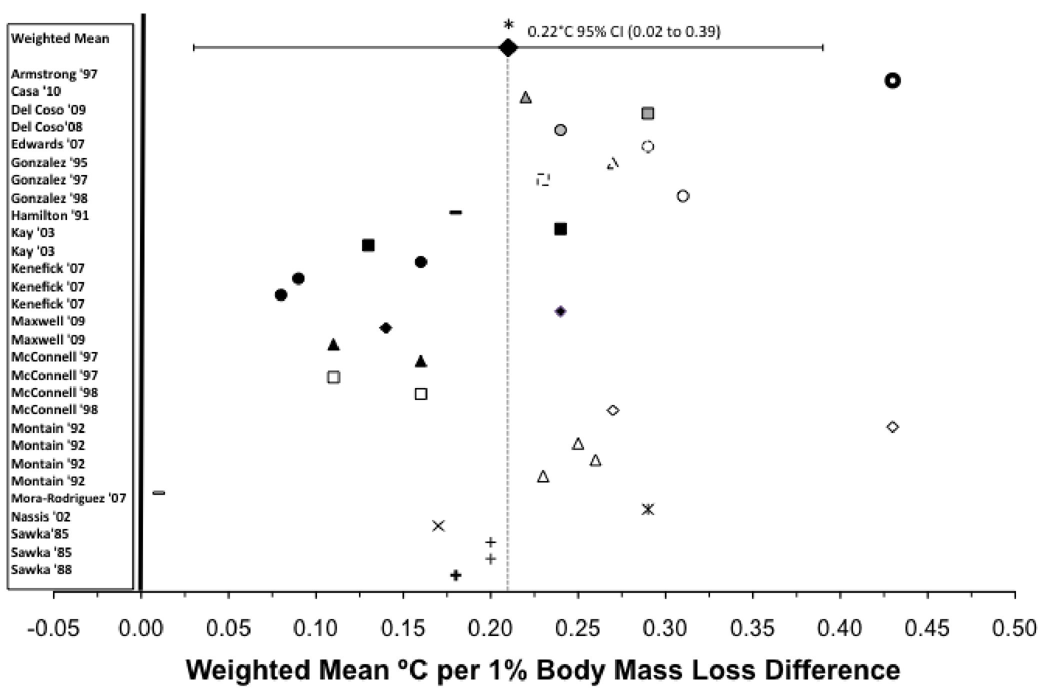


Figure 1.3

Even with many research studies demonstrating similar findings, there still appears to be some debate on whether or not the effects of dehydration on body temperature are associated.[39,40,64,65] One research study even determined that metabolic rate rather than dehydration is the main factor determining body temperature.[66] This is rather alarming given the large number of similar findings that have determined otherwise. One of the potential reasons as to why a discrepancy exists may have to do with the amount of control that is able to be garnered in the laboratory as opposed to the field setting.

Hormonal Control of Thermoregulation and Hydration

Hot environmental conditions have been well documented to have negative effects on the body's ability to thermoregulate and performance is often compromised.[1,42,47,67] Thermoregulation is closely tied to hydration status and the ability to regulate body temperature during exercise in the heat.[42,44] In order to better understand the interplay between temperature regulation and hydration we must first gain an appreciation for how hormones impact hydration at the cellular level.

Temperature regulation has a great influence on the hormonal control of cellular hydration. In order to accurately depict how body temperature impacts hormonal control we must first understand how body temperature is regulated. As discussed in Section A, body temperature is a carefully controlled variable that needs to be maintained within a narrow range.[68] Because of the influence of temperature on the rate of chemical reactions and enzymatic activity, thermoregulatory mechanisms through heat loss and gain heat must be regulated carefully by the hypothalamus.[69] Body temperature involves coordination of neural pathways that communicate with the hypothalamus, the brainstem, the spinal cord and the sympathetic ganglia. Within the hypothalamus the pre-optic anterior hypothalamic area (POAH) is primarily responsible for the coordination of this information.[70] Information can be sensed in two ways, centrally or peripherally.

Central Control:

Within the hypothalamus the POAH receives and integrates nervous activity from the skin and from blood compounds. Afferent information from neurotransmitters such as glutamate, GABA, corticotrophin-releasing factor (CRF), serotonin, the neuro-immune system and hypothalamic neurons all feed information to the POAH in a complex integrated system to regulate body temperature.[70] Glutamate is the primary neurotransmitter in the brain that sends the information from the periphery or the cutaneous thermal stimuli. GABA is an inhibitory neurotransmitter that is critical in sending efferent information to the thermoregulatory pathway. CRF is active in the presence of a fever and thermogenesis through prostaglandins and cytokines. Serotonin is closely linked to body temperature and usually increases in response to elevated body temperature. The neuro-immune system is closely linked to the thermoregulatory system in that immune function is closely linked to the hypothalamus. Furthermore, the immune cells share receptors for peptides, cytokines and corticosteroids. In addition, the immune system organs are also under the control of the hypothalamus and when the immune system is activated via the HPA axis, corticosteroids and neuropeptides give rise to phagocytic cells and lymphocytes, which are critical in the response to illness. The last piece of this system is the hypothalamic neurons. There are three different types of neurons in the POAH; warm-sensitive, cold sensitive and temperature insensitive.[71,72] Warm-sensitive are activated when the body temperature increases above 37°C [98.6°F] to begin the process of heat loss such as sweating and vasodilation of blood vessels in order to utilize the evaporative mechanism which is the body's most efficient mechanism of heat loss.[73] Cold-sensitive neurons increase their rate when the POAH senses temperature falling below the regulated range. These neurons result in the production of heat via repetitive muscular contraction or shivering. The third temperature insensitive neurons serve to assist both the warm and cold sensitive neurons.[74]

Peripheral Control:

The peripheral information from the skin is regulated by the neurotransmitters acetylcholine (ACh) and nor-epinephrine (nor-epi).[75] The tension of the muscles during contractions generates heat production as a byproduct of adenosine tri-phosphate (ATP) for energy. ACh directly controls this mechanism. In response to heat production in the periphery, sweat secretion is activated by the sympathetic cholinergic nerves.[76] Furthermore at the nerve endings nor-epi influences skin blood flow by activating nitric oxide which regulates autocrine and paracrine functions and helps in the vasodilation of blood vessels.[76,77]

Neuroendocrine Function Following Activation of the POAH:

Once the POAH is activated via the central and afferent peripheral mechanisms[71,76] in response to heat, there is an efferent response to increase the sweat response via sympathetic cholinergic nerves as well as to increase skin blood flow via dilation of vessels.[23,41,41] Many separate things are occurring simultaneously. The body is trying to increase blood flow to the skin to remove heat from the body while also trying maintain cellular hydration and blood flow to the exercising muscles. If blood flow is not delivered to the exercising muscles there will be a decrease in the delivery of substrates to the muscle and a reduction in the removal of metabolites. On the opposite side, if blood flow to the muscle is maintained there is a decrease in the delivery of blood

flow to the skin which decreases heat loss and increases heat gain in the tissues. (see Figure 1.4) This is the reason that we see multiple systems involved in this response. Due to the production of heat from the muscles, skin blood flow must increase to give the sweating mechanism the best chance possible to remove the heat from the body. In order to do so, fluid must be removed from the body and vaporized on the skin surface. Also blood flow to the muscle must be increased to allow for oxygen delivery used in aerobic metabolism. This movement of fluid to the skin and

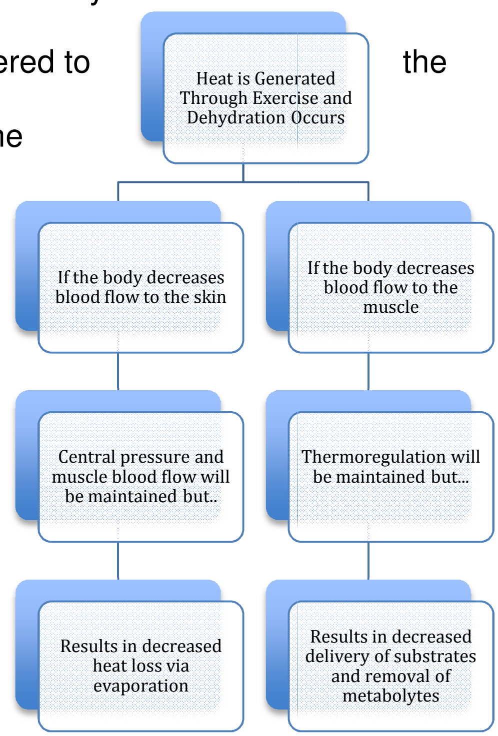
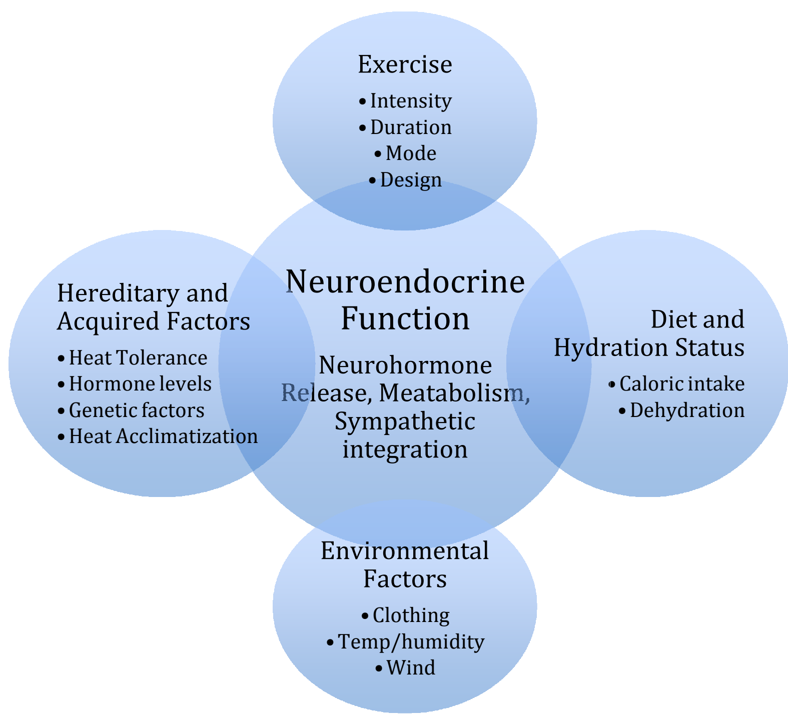


Figure 1.4 The Blood Flow Dilemma: Blood Pressure or Thermoregulation… Skin or Muscle?

muscles will have a direct effect on blood osmolality and blood volume. Both of these are factors are closely regulated within the body as stated in previous question. When osmolality and blood pressure changes osmoreceptors, kidney receptors, and cardiac receptors activate the release of anti-diuretic hormone (ADH) as well as the renin-angiotensin aldosterone and atrial natriuretic hormone (ANH) mechanisms respectively. All of these release hormones to regulate the fluid and sodium losses in the kidney in an attempt to maintain blood volume and the delivery of blood to the skin and exercising muscles.[41,43,78]

Factors Influencing Neuroendocrine Function:

Figure 1.5 Factors Influencing Neuro-endocrine Function



Neuroendocrine function is influenced by four things, 1) the exercise protocol, 2) the environmental factors, 3) the diet and hydration status and 4) the hereditary traits and acquired factors. (see Figure 1.5) They can be activated from sympathetic nerves directly or by the adrenal medullary hormones indirectly. When body temperature increases due to increased metabolic rate by the muscle, sweat rate, skin blood flow and heart rate must respond to maintain a homeostatic environment. Epi and Nor-epi must be released to maintain the cardiac function and the sweating response while simultaneous activation of removal of fluid from the kidney is conducted via renin, angiotensin II, aldosterone, ADH and cortisol. As metabolic rate continues to increase from peptides such as CRF, TRF, insulin, angiotensin, somatostatin, and the cytokines (IL-1α, IL-1β IL-6,IL-8, IFN-y, TNF-α) are released. These all directly respond in an attempt to maintain cell function when heat production is increased via exercise, environment, diet/hydration, and hereditary traits.

Factors that can aid significantly optimize the neuroendocrine function are diet/hydration, heat acclimatization and exercise training. By providing the appropriate caloric intake and electrolytes, the body will have the glucose and free fatty acids (FFA) it needs for the production of ATP and the maintenance of cell volume. Hormones and neurotransmitters may stimulate the mobilization or storage of substrates that will benefit the body. If the individual eats a diet that is not adequate, this may cause alterations in the neuroendocrine function and will increase stress on the body. This catabolic response will likely be indicated by circulating cortisol levels as well as other cytokines. Hydration status is also critical in optimizing the neuroendocrine function. With dehydration during exercise, the eccrine sweat glands are less sensitive and there is decreased sympathetic cholinergic stimulation via ACh and nor-epi. Hydration will allow for not only optimal cell volume but will maintain blood volume and help with skin blood flow and evaporative heat loss. Heat acclimatization will also help to optimize the neuroendocrine response. During heat acclimatization, increased nor-epi and dopamine levels in the POAH will occur. The neural networks will become accustomed to the imposed stress and will undergo neural plasticity. The CNS will become habituated to the stress being placed on in during the heat acclimatization process. Furthermore, enzymatic adaptations and organ responses will occur further aiding in the subsequent response. Rate limiting enzymes in the use of substrates or hormone production will increase in response to heat acclimatization. Organ responses such as lower resting body temperature, which is regulated by the hypothalamus, increased sweat threshold response, and sweat gland activity will all aid in thermoregulation. When heat acclimatization is conducted through the use of exercise training this will also increase the endocrine capacity, and as a result increased levels of ACTH, Epi, Nor-epi become tolerable.

Overall the adaptations that the body undergoes constantly have to do with the SAID principle or specific adaptations to imposed demands.[79] The body is continually becoming accustomed to the stresses that are put on it. Diet, training, heat acclimatization are all about adaptation to stress. Expose the body to the correct type, amount, frequency and duration of stress and the body will adapt. Many of the mechanisms that adapt are directly controlled via the neuroendocrine response. Training the neuroendocrine response is critical to performance during exercise. However, training too much or “over-training” or repeated amounts of stress either in sport or life can also significantly influence the neuroendocrine system.[80] Hubbard et al.[81] proposed the Energy Depletion

Model which demonstrates how the body can be placed in a vicious circle. When someone is experiencing a stressor it will undergo a stress response. There will be an increase rate of neurotransmitter activity, which will cause an increase in the activation of cells. This increase leads to a greater rate of metabolic processes, which in turn increases the rate of heat production.

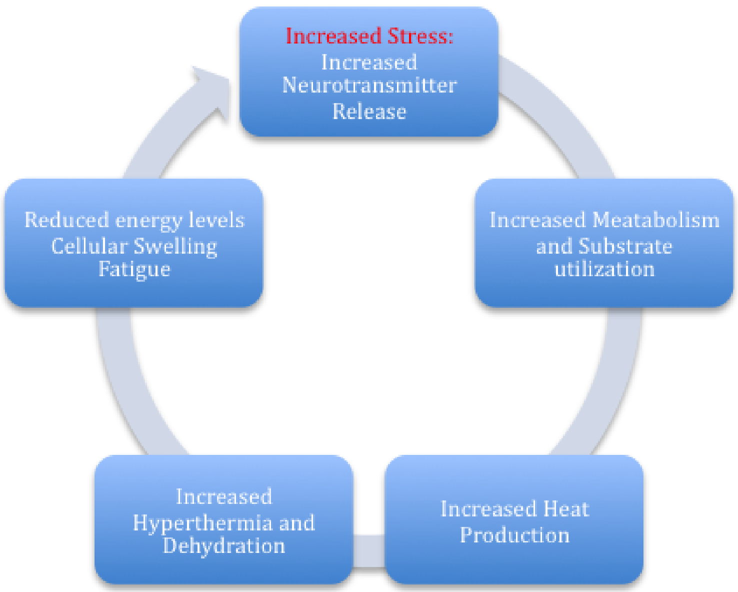


Figure 1.6: Stress-Induced Response During Exercise in the Heat & Energy Depletion

Energy levels as a result of the usage of stored metabolic substrates are reduced. Furthermore increased levels of dehydration and hyperthermia can be experienced. Cells begin to swell due to increased permeability and fatigue ensues. Unless hormonal responses can establish equilibrium, the body will continue in this circle. (see Figure 1.6) The endocrine system needs to maintain homeostatic environment and until the level or amount of stress is reduced on the body temperature and cellular hydration will be influenced.

In conclusion, the hormonal control of thermoregulatory mechanisms is well established and very much intertwined with the neural control (both central and peripheral) throughout the body. These same factors are very much related to the control of hydration and blood volume throughout the body. The overlap of these basic science mechanisms is critical for one to understand when attempting to conduct applied research and science. This understanding provides the foundation for prevention strategies to reduce body temperature both through the maintenance of hydration and cooling modalities.

Cooling Modalities and Hand Cooling

Cryotherapy has been utilized in all sport venues across the country and is well documented in the body of literature.[82–87] Various cooling modalities have been developed all with the goal of providing a physiological benefit to the athlete.[86] The purpose of these various modalities is to provide a method for the additional removal of heat from the body. In some cases, the rapid removal of heat from the body is critical to survival and proper treatment (i.e. exertional heat stroke (EHS))[88,89] and is recommended as best practice for healthcare providers in preventing sudden death in sport.[90] However, scenarios where EHS is not present and prevention of dangerous elevations in body temperature are important, other cooling methods as well as the timing of cooling have been examined.

The type and timing of cooling has been examined extensively for pre[35,57,86,87,91–112] and post cooling[88,89,89,113–121] as well as its effect on performance however little research exits in the area of cooling during exercise breaks.[11,103,122–130] Pre-cooling has garnered the most attention from researchers and coaches in an attempt to lower athletes resting body temperature prior to warm weather activity in an effort to allow for increase exercise duration and delay the onset of thermally induced fatigue. With pre-cooling, depending on the capability of the device to remove heat from the body, it may lower the amount of the subsequent heat stress. Another possible theory behind the effectiveness of pre-cooling is the reduction in perceived strain that the athletes experience.[102] Furthermore, pre-cooling may also assist in the redistribution of blood flow to the core and working muscles rather than to the skin to cool. This has been supported by reductions in heart rate in many studies.[35,91,95,96] Many methods such as water-immersion, [35,57,101,107,131,132] direct application of ice packs to the skin,[132,133] and wearing ice vests[93,95,98,100,110,112,132] have been reported in the literature. Readers should referred to a meta-analysis by Wegmann et al.[91] that specifically examined 18 studies determining the influence of pre-cooling on sports performance. (see Figure 1.7) Pre-cooling demonstrated large effects in hot and moderate environmental temperatures. Performance increases of 6.6% and 1.4% respectively for hot and moderate with endurance tests showing the greatest performance gain of 8.6%. In the same study, modalities such as cold drinks and cooling packs demonstrated the greatest change in performance with changes of 15% and 5.6% respectively. In another meta analysis conducted in 2012 by Tyler et al.[92] they too examined the effect of cooling prior to exercise on performance in hot (WBGT> 26°C) conditions. This meta-analysis revealed n= 28 articles (n= 23 prior to; n= 5 during exercise). Pre-cooling demonstrated a moderate effect (d= 0.73) on subsequent performance with intermittent and prolonged performance demonstrating greater effects than sprint performance which was impaired. (see Table 1.3) Local pre-cooling of various body parts has also been well documented during short term intense exercise performance.[134] Researchers have examined pre-cooling of the foot,[135] lower leg,[136] immersion of one or more legs,[137–140] forearm,[141,142] and ice bags/gel packs[143–145] on exercise performance during various performance tests. The findings from this review concluded that 57% (32 of 56) revealed decreases in performance and suggested that pre-cooling may in fact be detrimental to short term intense exercise and prevent the muscle from warming up to optimal temperature for force production.

Often even when adequate reductions in pre-cooling have been established, cooling during exercise is still required to ensure that the individual is able to accommodate the thermal strain. Methods such as ice vests[11,103,126] and cooling using a cooling collar[123–125] have been implemented and have shown promise. In the same meta-analysis previously mention by Tyler et al.,[92] cooling during exercise demonstrated a positive effect on performance and capacity (d= 0.76) and during exercise performed in compensable heat stress conditions, a smaller

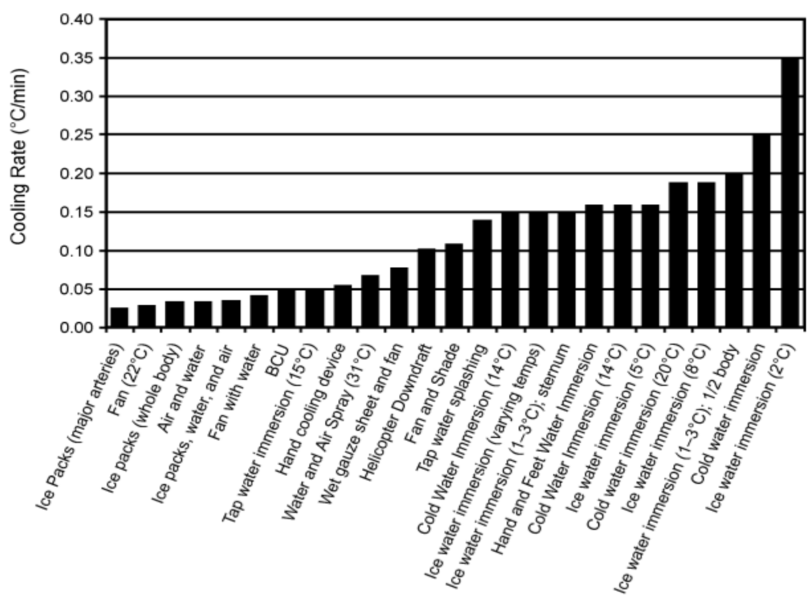
Table 1 Summary of the main study design details and data from the articles included in the meta-analysis which used cooling during exercise

Reference	Participants	Protocol	Ambient conditions	Cooling intervention	Effect size (d)	Main effect for physiological and perceptual variables during exercise: Area Cooled	Core temp	Skin temp	Heart rate	RPE	TS	Sweat rate
Ansley *et al*[34]	N=9 males	CE T_{exh} @ 75% $\dot{V}O_{2max}$	T_{amb}=27–29°C rh=40–60% WBGT=27°C	Head. Fan cooling with water misting	Cap: 0.51	↓*	↔	-	↔	↓*	-	-
Kenny *et al*[35]	N=10 males	TR T_{exh} (or 120 min) @ 3 m/h wearing NBC suit	T_{amb}=35°C rh=65% WBGT=38°C	Torso. Ice vest worn under the NBC suit	Cap: 2.26	-	↓*	↓*	↓*	↓*	↓*	-
Tyler *et al*[36] (A)	N=9 males	90 min TR TT_{pre}	T_{amb}=30°C rh=50% WBGT=29°C	Neck. Cooling collar	Perf: 0.29	↓***	↔	-	↔	↔	↓*	↔
Tyler *et al*[36] (B)	N=8 males	15 min TR TT_{pre}	T_{amb}=30°C rh=50% WBGT=29°C	Neck. Cooling collar	Perf: 0.23	↓***	↔	-	↔	↔	↓*	↔
Tyler and Sunderland [28]	N=8 males	TR T_{exh} @ 70% $\dot{V}O_{2max}$	T_{amb}=32°C rh=50% WBGT=31°C	Neck. Cooling collar	Cap: 0.43	↓***	↑*	-	↑*	↔	↔	↔
Tyler and Sunderland [37]	N=7 males	90 min TR TT_{pre}	T_{amb}=30°C rh=50% WBGT=29°C	Neck. Cooling collar	Perf: 0.67	↓***	↔	-	↔	↔	↔	↔
Tyler and Sunderland [37]	N=7 males	90 min TR TT_{pre}	T_{amb}=30°C rh=50% WBGT=29°C	Neck. Cooling collar (replaced at 30 and 60 min)	Perf: 0.62	↓***	↔	-	↔	↔	↔	↔

↑Increased compared with control; ↓ decreased compared with control; ↔ no change compared with control;—not measured.
*p=<0.05; **p=<0.01; ***p<0.001.
Cap, capacity; CE, cycle ergometer test; N, 6 rather than 5 because the manuscript by Tyler and Sunderland[36] contains two separate investigations (A and B); perf, performance; RPE, rating of perceived exertion; temp, temperature; T_{exh}, test to exhaustion; TR, treadmill test; TS, thermal sensation; TT, time-trial; TT_{pre}, preloaded time-trial; WBGT, estimated wet bulb globe temperature.

effect was observed (d= 0.45) than when UCHS was present (d= 2.26). Authors contributed this to the greater potential of the cooling modality to remove heat when the body is in an uncompensable situation compared to a compensable scenario. Other research in the area of cooling during exercise by Tyler and Sunderland[124,125] in two separate investigations examining neck and collar cooling during treadmill walking, determined that performance demonstrated moderate effects with cooling however no changes in body temperature or heart rate were observed and in another study core temperature and heart rate was actually higher in the cooling group compared to control.[123] Kenny et al.[11] also examined the influence of a torso ice vest on body temperature during exercise in the heat while wearing NBC clothing. Temperature, skin temp, heart rate, RPE and thermal sensation were all significantly reduced and improved exercise capacity. Ansley et al.[122] utilized head cooling as well as a misting fan and observed no changes in body temperature or heart rate. Local cooling is another area that has been examined during short term intense exercise again conducted in the review by Kwon et al.[134] Opposed to the pre-cooling findings, Kwon found that 100% of studies examining palm cooling[146,147] and ice packs over arm and shoulder between sets[148,149] demonstrated positive effects which has been attributed to the reduction in sensory perception and pain reduction and may be serving as a disinhibitory modality enabling maximal recruitment of motor units. Others suggest that the mechanism may be at the muscle spindle. Decreased nerve conduction velocity and muscle spindle activity may allow the muscle to generate more tension and thus a greater amount of force during eccentric activities. This increased slow twitch muscle fiber recruitment delays immediate activation of the fast twitch fibers permitting slow twitch to contract longer before fatigue develops.

Post exercise many modalities have been compared. In a systematic review examining the rate of acute cooling modalities McDermott et al.[150] examined twenty-five various methods of cooling to find that only studies with water immersion ranging from cold 14℃ to 2℃ demonstrated acceptable cooling rates ≥ 0.15℃/min. (see Figure 1.8)



One study conducted by DeMartini et al.[86] examined the effectiveness of 10 different cooling modalities on core body temperature. Various cooling modalities were applied after 45-60 minutes of exercise and demonstrated that only a few of the cooling modalities resulted a rapid drop in core body temperature than the control trial. The control group had a 1.50˚F drop in body temperature in 17 minutes compared to the same 1.50˚F drop using three other methods CWI, Nike Ice Vest™, and ice bucket cooling of hands and feet which were 14.0, 16.0, and 12.0 minutes respectively. The remainder of the cooling modalities ice towels, GameReady™ Vest, Rehab Hood™, and Emergency Cold Contaminant System™ (ECCS), Port-a- cool™ fan, and sitting in the shade all had decreased cooling rates compared to the control group. This study was the first to investigate most these cooling modalities simultaneously and formed a framework of understanding for potential tools that can be utilized to limit the amount of time individuals are subjected to physiological stressors.

Inflammatory Cytokines Response to Heat

Cytokines and Heat Stress:

Cytokines are intracellular peptides critical to proper inflammatory response, repair, and adaptation are released into the circulation in response to a stressor. Most commonly cytokines include interleukin (IL)-6, IL-12, IL-1β, IL-1ra (receptor agonist), IL-8, IL-10, IL-2, and tumor necrosis factor alpha (TNFα) among others. Cytokines can be further divided into pro-inflammatory and anti-inflammatory and work in concert to modulate the magnitude of the inflammatory response. IL-6, IL-10, IL1ra, and TNFrI & II are considered anti-inflammatory in nature while IL-1α, IL-1b, IL-2, and TNFα have been observed as pro-inflammatory. The balance between the pro- and anti-inflammatory responses is critical to ones response to stressors involving exercise, hyperthermia or the combination of the two.

Exercise associated hyperthermic stress and the activation of both humoral and cellular mechanisms of the immune system has been well established.[151–153] The role of cytokines has been examined extensively during controlled laboratory exercise in the heat[151,154–157] and furthermore in field research examining soldiers[158] and marathoners.[155,156] The physical exertion and prolonged stress associated with these activities initiates an inflammatory response very similar to that observed with a soft-tissue injury or a simple infection. These mediators known as cytokines are released in an attempt to maintain the homeostatic environment of the cell and prepare for future stress induced responses.

When climatic heat stress or exertional heat stress is too great, the imbalance of the acute inflammatory response may lead to a systemic inflammatory response as described by Bouchama and Knochel.[151] Many often wonder what factors may predispose an individual to exertional heat stroke. Rav-acha et al.[159] examined 6 cases of fatal exertional heat stroke that occurred in the military and determined the major factors common to each case. He determined that exertional heat stroke often occurred in individuals who are unacclimatized, dehydrated, suffer from sleep loss, suffer from some sort of illness, or exercise at a level unmatched to their physical fitness.[159] These factors however fail to describe the processes within the body and how exactly one succumbs to EHS. Research in hyperthermic humans both EHS and non-EHS individuals as well as animal models has enabled researchers to examine this more in-depth. During exercise in the heat the body shunts blood to the periphery and to the exercising muscle in an attempt to meet their demands to both keep the body cool and muscles pumping. With a limited supply of blood, the body shunts the blood to these areas reducing splanchnic flow.[160] This reduction in flow leaves the gut ischemic in order to preserve blood pressure causing the gut to become leaky.[161] Gram negative bacteria normally contained within the gut and the lumen, cross the junctions and make their way into the circulation and can lead to endotoxicity[162,163] which the liver must now attempt to remove from the body.[164,165] These endotoxins increase release of inflammatory cytokines as previously described and prompt intravascular coagulations to occur. Furthermore, the release of endotoxins can lead to acidosis, impaired liver function,[164] and ensuing endoxemia. In situations related to excessive exercise associated hyperthermia (e.g. exertional heat stroke) this endotoxic response may lead to encephalopathy, renal failure, rhabdomyolysis, cerebral ischemia, myocardial infarction, and subsequent mortality if left untreated. Leon et al.[166] have accurately depicted this mechanism in a recent publication describing the systemic inflammatory response represented in the figure below.

Figure 1.9

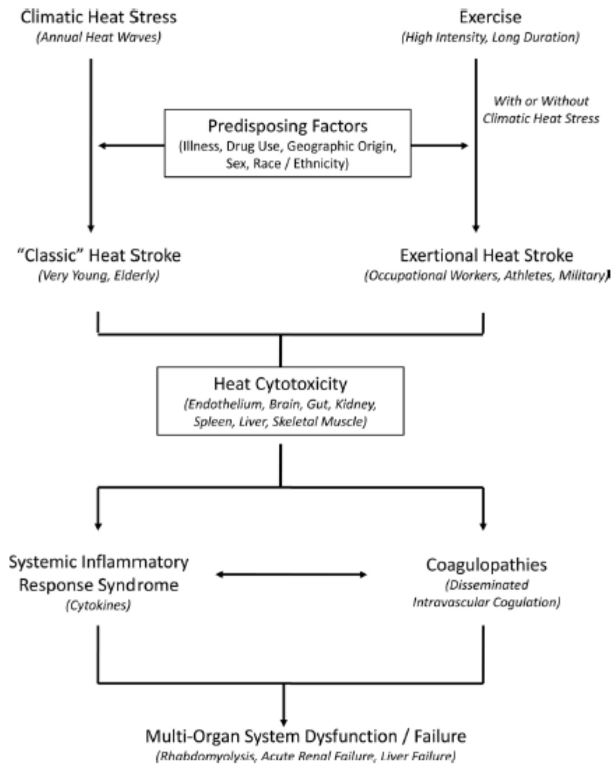


Fig. 1. Summary of environmental and predisposing factors that interact to cause "classic" (passive) and exertional heat stroke. Classic heat stroke is commonly observed in very young children or the elderly during annual heat waves. Exertional heat stroke is observed in young, healthy individuals that are performing a high rate of metabolic work in temperate or hot conditions. Predisposing factors, such as illness, drug use (e.g., anticholinergics, antidiurectics, ecstacy), geographic region of origin (Northern>Southern), sex (women>men), and race (Caucasian>African American) are confounding factors that increase the incidence of both forms of heat stroke. Following heat stroke collapse, heat cytotoxicity to the brain and peripheral organs initiates the coagulation cascade and a systemic inflammatory response syndrome that may culminate in multi-organ system failure and death.

It is not impossible for the liver to recover from exertional heat stroke but is very unlikely. To give an example Giercksky et al.[165] present the case of a 5km runner who suffered from severe liver damage with extremely high alanine aminotransferase (ALT) 48 hours later with a biopsy revealing extensive liver necrosis 5 days later. The patient was to have a liver transplant but remarkably recovered completely with conservative treatment. This is not the norm, patients commonly succumb to the systemic inflammation within 24-48hrs post-collapse.

Cases such as this one, has lead heat stroke researchers to further examine this response *in vivo*.[167–170] A great deal of this research in the last 15 years has focused on the human inflammatory cytokine response.[152,153,158,167,171] With the advancement of technology and assay kits equipped with the ability to detect a large number of markers simultaneously this has become more common. One landmark study in 1993 by Bouchama et al.[171] sparked this focus when they examined exertional heat stroke patients in pilgrimage to Mecca. IL-6, IL-1 β, and INF-gamma concentrations were observed and determined to be elevated in 100%, 39%, and 50% of patients respectively. Furthermore, IL-6 concentrations correlated with severity of illness ($r = 0.516$, $p = 0.03$) which has also observed in mice and has been theorized to be critical to the systemic inflammatory response syndrome (SIRS) response.[167] In a similar study also during pilgrimage,[172] TNFα, IL-1α , and lipopolysaccharide (LPS) were all elevated in acute exertional heat stroke (EHS) patients compared to controls. One very unique aspect of this study is they also observed these cytokine levels to decrease significantly following acute cooling prompting a great deal of research in this area. Other markers such as IL-8 and TNFα have been correlated with the clinical severity of EHS in a case report of 8 patients.[153] Furthermore, IL-1β was found to be correlated to body temperature and the cooling duration.

Cytokines and Exercise:

In an effort to gain a great appreciation for the response of cytokines during various types of exercise, researchers have examined the role of cytokines in during prolonged exercise,[154–156,172] brief exercise,[173] and sub-maximal exercise[157] in various conditions.[174] Ostrowski et al.[154] examined the cytokine response in runners before, immediately after, and in the hours following the Coppenhagen Marathon in 1998. They observed elevations in many markers including IL-6, TNFα, IL-1β among others as depicted below in Figure 1.10. The cytokine response has also been compared in trained and untrained men[175,176] (Figure 1.11). Various cytokines such as IL-1α, IL-1β, IL-6, sIL-6R, TNFα, IL-8, IL-10 and IL-12p70 have all been examined on multiple occasions. Table 1.4 is adapted from Heled et al.[152] to just report those cytokine studies in humans under heat stress.

Figure 1.10

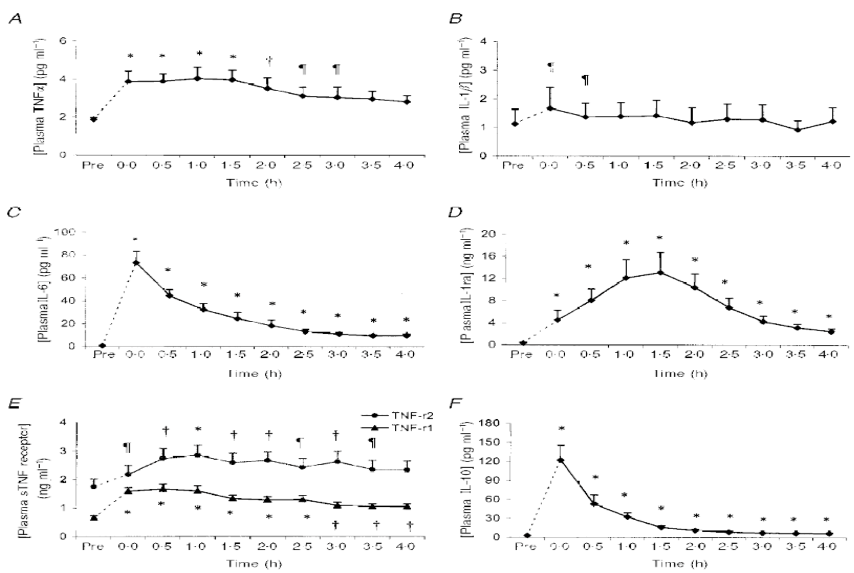


Figure 1. Plasma cytokine concentrations measured before (Pre) and every half hour in the 4 h resting period after a marathon race

Data are plotted as geometric mean with 95% confidence interval (c.i.) (IL-1β, IL-6, IL-1ra and IL-10) or as mean with 95% c.i. (TNFα and sTNF receptors). Legend denotes difference from the pre-exercise value in the Tukey multiple comparison test ($*P < 5 \times 10^{-5}$; $\dagger\ P < 5 \times 10^{-3}$ and ¶ $P < 0{\cdot}05$, respectively).

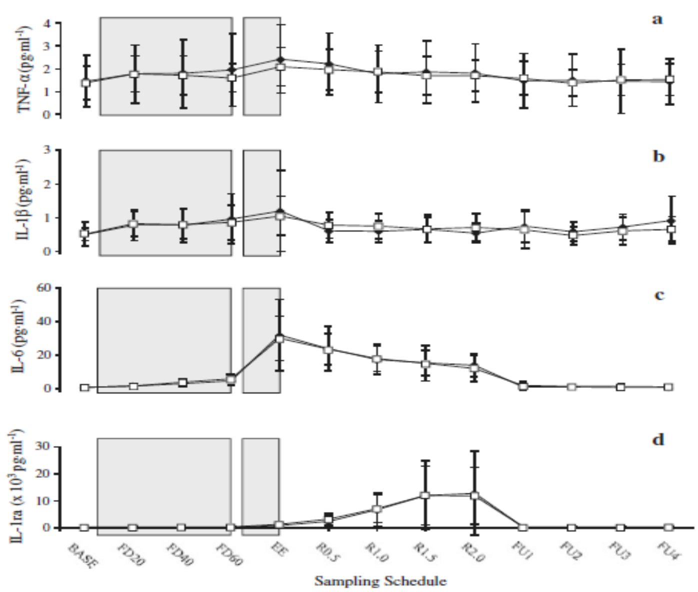
Table 1.4

Cytokine Responses To Heat Stress In Humans

Marker	Inflammatory Role	Response to EHS or Stress	Reference
IL1	Pro-Inflammatory	Increased	[172,177]
IL1	Pro-Inflammatory	Increased or No change	[153,158,171,177,178]
IL6	Pro-Inflammatory Anti-Inflammatory	Increased	[153,158,168,171,177,178]
TNF	Pro-Inflammatory	Increased or No change	[153,154,156–158,172,177]
IL8	Pro-Inflammatory	Increased or No change	[156,158,174,178,179]
IL10	Anti-Inflammatory	Increased	[154,158,178-180]
IL12p70	Pro-Inflammatory	Response Not Assessed To Date	

Figure 1.11

Fig. 3 TNF-α (**a**), IL-1β (**b**), IL-6 (**c**) and IL-1ra (**d**) concentrations before (*BASE*), during (*FD20–FD60*) and for 2 h after exhaustive running (*EE–R2.0*), and on four follow-up days (*FU1–FU4*) in the RA (*open squares*) and ET (*filled diamonds*) groups. Values are mean ± 1SD. LMM revealed the following significant main effects of *Time* for TNF-α, IL-1β, IL-6 and IL-1ra (all $P < 0.001$), but no significant *Group* × *Time* interaction for any variable (TNF-α, $P = 0.168$; IL-1β, $P = 0.118$; IL-6, $P = 0.708$; IL-1ra, $P = 0.468$)



In all heat stress studies to date, with the exception of IL-12p70 have shown an increase or no change in response to heat stress or EHS. Elevation in some of these cytokines, (e.g. IL1α, IL-6, and TNFα) has been shown to promote the increase in body temperature and are considered pyrogenic in nature. That being said, cytokines that are both pro-, anti-, and a combination of both pro- and anit-inflammatory in nature have been found to change in response to heat stress or EHS within the circulation. Based on this compilation of cytokine research in human subjects, research in the areas of IL-12p70 during heat stress is lacking and furthermore only a few studies have examined cytokines post treatment intervention following heat stress.[168,171,178]

Cytokines and Cooling:

The cytokine response to cooling following heat stress has only been examined in patients admitted for heat illness during pilgrimages to Mecca. To date, only one study by Rhind et al.[181] has examined the effect of attenuating the rise in body temperature while cycling in a water bath on circulating cytokines. This process described as “thermal clamping” was found to successfully terminate increases in circulating cytokine concentrations and the stress hormones epinephrine, nor epinephrine and cortisol. No research to date has examined the influence of any other cooling modality during exercise and examined the cytokine or stress hormone response.

One type of cooling of recent interest is that of peripheral cooling of the hand. Cooling of the hand has focused a great deal on physiological variables such as body temperature,[182–186] perceptions of fatigue,[134,146,147] and performance,[184,187–190] however no research to date has examined the effect of palm cooling on the inflammatory process more specifically cytokines and markers of stress. Another area where cooling research through the hand is lacking is during uncompensable heat stress. Cooling during heat stress in general may be one of great potential areas for research related to inflammatory cytokine response. This applies not only to peripheral hand cooling modalities, but all other types of cooling. It would be quite interesting to examine not only the influence of cooling on mitigating the inflammatory response, but also to determine if there is a dose-response relationship similar to the thermal clamping seen in the studies by Rhind et al.[181] Another potential area not yet examined is the role of both hydration and cooling, two current strategies utilized in sport, on mitigating the inflammatory response. The effects of hydration and cooling on mitigating cytokine response to prevent excessive heat-related release, improve recovery, and performance would be of great value to players, coaches, and the sports medicine staff.

Currently, excessive markers of inflammation and cytokine release has been observed pilgrimages,[168,172,178] marathons,[154,155] and even football.[191,192] If we are to prevent this overwhelming response during hyperthermia and exercise stress from occurring in sport, we must first continue to quantify the extent of the inflammatory response during controlled laboratory studies. Furthermore we must quantify during both compensable and uncompensable heat stress as well as explore various methods for maximizing heat loss to prevent dangerous elevations in body temperature such as cooling.

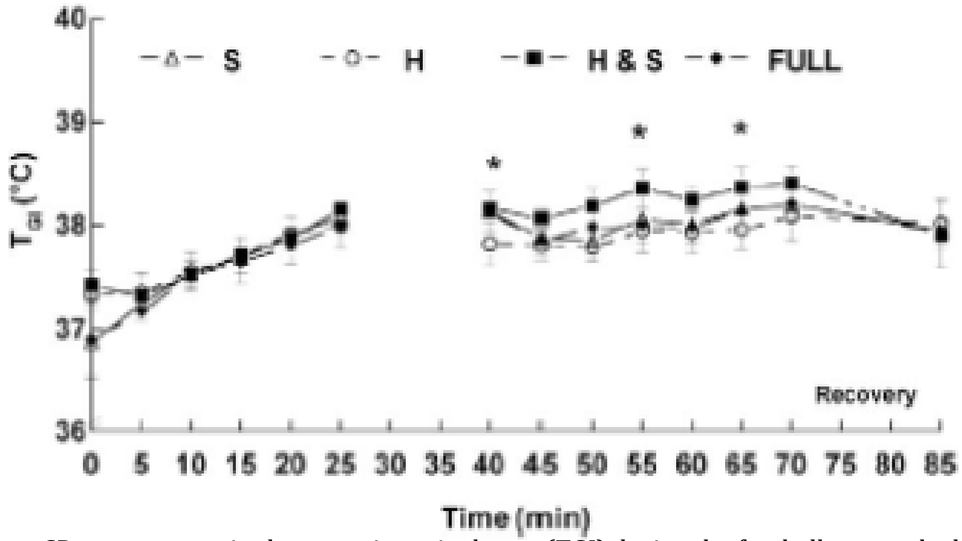
Cooling During American Football

American Football presents a challenge to the body's ability to thermoregulate. The uniform and equipment minimize the skin surface area of available for sweat evaporation.[17,18,20,21] Couple this with large body mass individuals[40,193–195] particularly linemen (87% of whom are classified as obese),[37] intermittent high-intensity exercise, and a season that begins in the hottest and most humid month of the year,[24,35] and the risk for heat illness increases drastically. As a matter of fact the risk of death from exertional heat stroke (EHS) in the United States is highest in the sport of high school football,[196] especially during the month of August when preseason begins particularly during the first 3 weeks of August when 2-a-days often begin.[24] Intensity and equipment are likely the greatest factors during exercise it the heat that result in significantly more heat stress than other sports[36] and will be the focus in the subsequent sections.

The intensity of football is largely the greatest heat-producing element in football. Although not continuous, the high intensity play during a football game or practice places high demands on the energy systems of the body. Football is often dominated by short bursts of intensity activity normally lasting 2-5 seconds in duration. Following these intense bursts, brief recovery between plays often occurs for 20-30 seconds before the ensuing play. This relatively short rest duration has been shown to be insufficient for the restoration of phosphocreatine stores[197] and the reduction of heat production by the exercising muscles.[38] The ability to optimize evaporation with the confines of the game is also a factor closely tied with the intensity of play. Positions that perform less running and in close proximity to other players (e.g. linemen) appear to have a reduced potential for evaporative heat loss. Recent research in NCAA football players has elucidated this when they examined the air flow and heat loss potential for lineman vs. non-linemen. They determined that linemen demonstrate a decreased ability to self-generate air flow due to the more static nature of their activities[198] and thus have a reduction in heat loss potential. This information supports the notion that both intensity and positional differences impact the delicate balance between heat production and heat loss and therefore breaks become extremely vital to recovery and heat loss or so you would think. Contrary to what one would assume, it has been shown that during the rest periods in football body temperature does not decrease, instead it plateaus then continues to rise again when the intensity of exercise increases.[38] This information supports the impetus for cooling even more during breaks in this highly intense sport.

The equipment is another challenging aspect unique to football and greatly limits the amount of heat loss that the body is capable of. The microclimate that is created between the equipment and the skin is not conducive to evaporation and instead the sweat remains on the surface of the skin and fails to evaporate. Unless sweat evaporates from the body, no heat is actually removed. Hitchcock et al.[19] confirmed this in their investigation of the energy cost of offensive lineman (OL) during a simulated football practice while wearing four separate combinations of football equipment. During the testing session subjects performed simulated football drills and determined that body temperature increased the most while wearing helmet and shoulder pads and that the metabolic cost (>50% VO_2max) was greater than previously determined.

Figure 1.12



Mean ± SD temperature in the gastrointestinal tract (TGI) during the football protocol while wearing different football ensembles (S= shorts only, H= helmet only, HS = helmet and shoulder pads added, FULL= helmet and shoulder, thigh, and hip pads added). *Significantly higher value ($p<0.05$) for HS compared to H.

Another study by Armstrong et al.[20] further quantified the increase in heat stress while wearing an American Football uniform. In this study they controlled exercise intensity and measured physiologic strain to investigate critical internal temperature during exercise with full pads, partial pads, and a control condition of no pads (Figure 1.12). In this study, subjects wearing full football pads when compared to partial pads, or control, experienced uncompensable heat stress as indicated by the continuous rise in body temperature rather than plateau observed in the other conditions. This study and others demonstrate that protective equipment can allow football players reach a state of uncompensable heat stress quicker than most athletes.[18,21] It has been determined that while wearing full football equipment, uncompensable heat stress can occur in as little as 26°C and 52% relative humidity.[19] With this information in mind athletic trainers, coaches, and players need to continue to examining ways to combat the heat gain inherent to the sport of football. Research to prevent heat gain and associated heat illness has focused on lowering body temperature during breaks[126] and during games[103] through the use of cooling modalities. This too has been met with challenges.

Cooling opportunities in the sport of American Football can be a logistical challenge to the sports medicine team, coaches, and more importantly the players. Football traditionally consists of four quarters (15 minutes each) with a half-time following the second quarter of 20-30minutes. Cooling has traditionally been performed on the sideline and during the half through the use of ice-towels, cold IV, removal of equipment, and ice bag application to various parts of the body. The inherent problem with American football is that equipment covers most of the body surface area, limiting the cooling potential. The large the surface available to cool, generally the larger the cooling rate.[89,118,150] So as healthcare providers and coaches we are left to deal with the following potential areas to cool during breaks and half-time: the head, neck, forearm, and hands. The unfortunate news is that the cooling rates when applied to these areas using a variety of modalities is not as effective[86,150] in the removal of heat. Also given the time restraint and the implementation of whole body cold water immersion may not be feasible given the large number of players participating. Therefore, we must continue to investigate cooling in these various areas of the body to examine if there is in fact a more efficient technology that can remove heat effectively and reduce body temperature.

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CHAPTER 2: THE EFFECTS OF INTERMITTENT HAND COOLING ON INTERNAL BODY TEMPERATURE AND EXERCISE PERFORMANCE DURING HIGH SCHOOL FOOTBALL

Abstract: American football equipment challenges ones ability to thermoregulate during exercise. Whole body cooling modalities are difficult to implement and peripheral cooling modalities are yet to be investigated in the field setting. Furthermore, it is unclear if reduction in body temperature will lead to performance gains. **Objective:** To determine the effect of intermittent hand cooling during practice breaks on body temperature, perception, and performance. **Design:** Matched pairs. **Setting(s):** Field research at two separate Arkansas high school (HS) football teams. **Participants:** HS#1: Twenty-six males (age=16±1yrs, height=179±6cm, body mass=87.58±18.69kg, %body fat=18.9±9.8%) HS#2: Thirty males (age=17±1yrs, height=181±6cm, body mass=89.52±14.68kg, %body fat=22.7±8.7%). **Intervention:** Participants at each HS were matched and randomly allocated to the hand cooling (HC) group or the control (CTRL) group. During breaks, the HC group received cooling during practice while the CTRL did not. Both groups were permitted to drink fluid ad-libitum. **Main Outcome Measures:** Gastrointestinal temperature (T_{GI}), heart rate (HR), body mass, rating of perceived exertion (RPE), thermal, and thirst sensation was recorded before (PRE), during breaks, and after (POST) exercise. %body mass loss (%BML) and pain was measured PRE and POST. During practice, total distance, Player Load™ (PL), repeated high intensity efforts (RHIE) bouts, maximum velocity (V_{max}), exertion index (EI) and velocity zones were recorded using GPS accelerometry. Separate one-way repeated measures ANOVA with post-hoc Bonferroni corrections were utilized to examine differences ($p < 0.05$). **Results:** Participants in the HC and CON groups at both HS#1 and

HS#2 were not different ($p>0.05$) for any matched demographic criteria. %BML over all days was not different between HC and CON at HS#1 or HS#2 ($p= 0.562$; $p= 0.982$). A significant increase and main effect for time from PRE to POST was observed for T_{GI} at both schools on all days (p • 0.001). Moderate effects were observed for HC (ES=0.65) on Day 2 in HS#2 although •T_{GI} was not significantly at HS#1 ($p= 0.07$) or HS#2 ($p= 0.782$). Avg HR and HR_{max} were moderately elevated on Day 3 (ES= 0.55; ES= 0.71) respectively. A significant group x time interaction was observed for thermal sensation at HS#2 on Day 3 ($F_{1.14}=5.508$, $p=0.034$, $\eta^2= 0.282$, observed power= 0.589) with HC perceiving greater thermal sensation at POST than CON ($p=0.024$). RPE POST and during practice were moderately decreased on Day 1 and 4 (ES= 0.69, 0.74) respectively for CON in HS#1. Pain was significantly higher for CON ($p=0.004$) on Day 2 at HS#2. Moderate effects for HC were observed for RHIE bouts and distance covered in velocity zone 2 (ES= 0.77; ES= 0.62) on DAY 2 for HS#1.

Conclusion: Hand cooling with negative pressure during pre-season football practice demonstrated significant differences in thermal sensation and pain with moderate effects on different days for T_{GI}, HR, RPE, RHIE bouts and distance covered in velocity zones. These findings suggest that hand cooling has some effect and serves as a foundation for future research under more extreme environmental conditions.

INTRODUCTION:

During exercise in the heat, especially in hot and humid environments, the risk for exertional heat illnesses increases dramatically. Common types of exertional heat illness (EHI) include heat exhaustion, heat cramps and exertional heat stroke. Heat cramps and heat exhaustion are often easily combated with fluid replacement, correction of electrolyte imbalance, rest, and removal from the activity and/or heat source. Exertional heat stroke (EHS) on the other hand has the potential to be fatal and occurs when the body's internal temperature reaches •104°F or 40°C and is coupled with central nervous system dysfunction.[1–4] EHS is very much preventable and 100% survivable if rapidly cooled within the first 30 minutes from the point of collapse.[4,5] If we can prevent EHI from occurring and be cognizant of the factors that increase the risk for EHI, we can enable athletes to exercise safely and even improve performance. In order to gain an appreciation of the factors that increase the risk for EHI, we must first understand how the human body thermoregulates during exercise in the heat.

Much of the internal heat produced is generated from the work performed by the exercising muscles. When the muscles perform work, heat is the by-product and results in an increase in body temperature.[6] This increase in activity of the exercising muscle increases the demand for oxygen and nutrients which increase the need for the delivery of blood to the muscle. Concurrently, the same blood is required at the skin to remove the heat produced. This supply and demand dilemma presents a great challenge for the thermoregulatory system especially when exercising in hot and humid conditions.[7,8] When temperature increases within the body, the body turns to its most efficient mechanism of heat removal, evaporation of sweat from the skin.[9,10] Sweating enables the body to continue to exercise by effectively removing the heat from the body through evaporation. When internal heat is effectively removed during exercise in the heat, this is known as compensable heat stress. Conversely, in situations where evaporation is hindered (e.g. football equipment) or impaired (e.g. low total body water) body temperature continues to rise with no sign of plateau. This continuous rise is known as uncompensable heat stress.[6,11–14] Uncompensable heat stress situations occur when the evaporation required to maintain body temperature exceeds the body's maximum ability to remove the heat and thus heat is stored in the body and may elevate to dangerous levels.[6] Sports, particularly American football, can easily place the body into an uncompensable heat stress scenario that can lead to dangerous elevations in body temperature.[11,15–17]

The American football uniform presents a challenge to the body's ability to thermoregulate and minimizes the surface area of the skin available to evaporate sweat.[11,17–19] Couple this with large body mass individuals[20,21] particularly linemen (87% of whom are classified as obese),[22] intermittent high-intensity exercise (work), a season that begins in the hottest and most humid month of the year, and the risk for heat illness increases drastically. As a matter of fact, the risk of death from exertional heat stroke (EHS) in the United States is highest in the sport of high school football[23] during the first 3 weeks of August when 2-a-days often begin.[16] In an attempt to prevent deaths from occurring at the high school level, recommendations from the National Athletic Trainers' Association (NATA) for pre-season heat acclimatization guidelines have been implemented by many states and organizations.[24] These recommendations focus on phasing in equipment, exercise intensity, total exercise time in the heat, and contact over a period of 7 days. Recommended prevention strategies such as unlimited access to fluid and preparation of treatment modalities in the event of a heat illness are also included. Furthermore these guidelines create recommendations for rest breaks and even practice termination based on wet-blub globe temperature (WBGT). Twelve states have even implemented policy changes based on these guidelines since 2010 to ensure that heat acclimatization occurs during the first 7-10 days of pre-season football and results have shown that there is some promise it is working. In these twelve states, not one death from EHS has occurred since the passing of this legislation. Unfortunately, even with numbers decreasing in these 12 states, deaths from EHS are still present in 38 other states and will remain present as long as athletes do not acclimatize to exercise in the heat, follow recommended guidelines, and remain uneducated on ways to prevent it from happening. This was made evident in a 2013 report from the National Center for Catastrophic Sports Injury Research that in the past 10 years EHS is showing little to no signs of slowing with 31 deaths from 2002-2012.[25] This is increased compared to the previous 10 years where the number of EHS deaths was 21 from 1992-2002.[25] Furthermore another study by Kerr et al.[23] demonstrated that in a survey of 1142 of athletic trainers providing care to high school football athletes, 20.3% reported treating at least one EHS event.[23] With the risk for heat illness remaining, athletic trainers, coaches and players are examining ways to prevent EHI by lowering body temperature during practice[26] and during games.[27]

Various methods of cooling have been demonstrated in the literature to effectively reduce body temperature acutely[28–33] with ice water and cold water immersion providing the greatest cooling rates.[28] The inherent difficultly with ice water immersion between breaks or during regularly scheduled breaks in sport (e.g. halftime in football) is that it lacks feasibility in equipment laden sports and requires a great deal of resources to cool an entire team for sports with large numbers of athletes. Various "sideline conducive" but less effective cooling modalities have been examined such as ice packs at the head, neck, and axilla[31] fanning cold water immersion of the hands and feet,[34] cooling vest,[26,35–37] large misting fan[37] cooling hood[37] and ice towels.[37] DeMartini et al.[37] examined 10 of these cooling methods and found that many of these modalities (large misting fan, head cooling, ice towels, and two types of cooling vests) were no different than sitting in the sun. One modality however, forearm and lower leg immersion in an ice bucket was significantly different and had a cooling rate of 0.074°C·min^{-1} which was higher than cold water immersion 0.065°C·min^{-1} after 10 minutes of cooling. This raises an interesting theory about cooling of the periphery, specifically of the hands and feet.

Previous research examining the blood flow to the non-glaborous (non-hairy) surfaces such as the hands and feet suggest that in hyperthermic individuals that normal vasoconstriction does not occur when hands are placed in cold water and that cooler blood is delivered directly to the core via superficial veins of the periphery.[38,39] The delivery occurs when structures called arteriovenous anastomoses between peripheral arteries and veins are open and permit the flow through the peripheral veins [40,41] With this anatomical possibility in mind, cold has been applied to the surfaces of the hand have been investigated in depth and have shown that it is an efficient method to reduce body temperature especially when negative pressure is applied to the hand.[42–48] Typically this is done utilizing ice baths (IB) to submerge the hands and it was often studied as a treatment tool for EHI[37] although there are studies that have investigated its effect on body temperature either as a pre-cooling modality[48] or post exercise.[34,44,49,50] Therefore it is feasible that peripheral hand cooling may mitigate the rise in body temperature through heat extraction from the hand. To date no research in the area of American football has been conducted investigating peripheral hand cooling in the field setting using a negative pressure device. Therefore, the purpose of the present study is to examine the influence of peripheral hand cooling using a commercially available device on body temperature, performance and perceptual measures during preseason football practice.

METHODS:

Experimental Approach to the Problem:

American football imposes a unique physiological stress due to the size of the individuals, the equipment worn, the timing of pre-season, and the high-intensity of the activity. These factors are exacerbated when athletes are not acclimatized to exercise in the heat as recommended by the most recent Inter-

Association Task Force on Preventing Sudden Death in Secondary Schools.[51] Furthermore, although it is well known that reductions in body temperature result in increased performance when exercising in the heat, football creates some challenges to efficient cooling. The protective equipment worn during football covers much of the body surface area and breaks are often too short for efficacious cooling in football. Common cooling modalities utilized in football currently consist of large misting fans and ice-cold towels, but the cooling rates are minimal given the short amount of time between plays. In an attempt to examine the ability for a newly developed peripheral hand cooling device with negative pressure to reduce body temperature and improve performance, an observational field study at two separate high school football programs near Fayetteville, AR were examined. Currently no research exits examining this device in high school football. Furthermore, to our knowledge this is the first time that high powered global positioning satellite (GPS) devices were utilized during pre-season high school football to quantify activity.

Participants:

We recruited football players from two separate Fayetteville, Arkansas area high schools. High school #1 (HS#1) consisted of twenty-six males (age: 16± 1yrs, height: 179± 6cm, body mass: 87.58± 18.69kg, % body fat: 18.9± 9.8%). (see Table 2.1) High school #2 (HS#2) consisted of thirty males (age: 17± 1yrs, height: 181± 6cm, body mass: 89.52± 14.68kg, % body fat: 22.7± 8.7%). (see Table 1.2) At both high schools players were matched to another individual from their own team based on the following demographic criteria (position, body mass, age, and height). Players were then randomly allocated to groups via coin-flip. At HS#1, n=13 were allocated to the hand cooling group (HC) and n=13 were allocated to the control (CTRL) group. At HS#2, n=15 were allocated to the HC group and n=15 to the CTRL. All participants completed a medical history questionnaire and were excluded if any of the following criteria were met: (1) previous history of exertional heat stroke in the last 3 years, (2) intolerance to the heat, (3) contraindications for the use of the gastro-intestinal pill, (4) cardiovascular, metabolic or respiratory disease, and (5) medication or dietary supplements known to alter thermoregulation. All participants/parents provided written informed consent and research was approved by the Institutional Review Board at the University of Connecticut for Human Studies.

Familiarization and Baseline Testing:

The day prior to testing all participants performed baseline testing. Participants were familiarized to the testing protocol and given detailed instructions about the perceptual scales utilized before, during and after practice. Participants were guided through the sequence of events that would occur before, during, and after practice each day. First participants were asked to provide a urine sample into a clean, inert, plastic container. Hydration status was assessed using a urine color chart (Ucol) and urine specific gravity (Usg) using a hand held refractometer (Atago 300 CL, Atago, Japan). Height was assessed using a standard measuring tape (Stanley Leverlock, Black and Decker, New Britain, CT) and body mass was obtained using a calibrated scale (model BWB-

800A; Tanita Corp, Tokyo, Japan). A single researcher performed three site skin-folds measurements on all athletes using skin fold calipers (Lange, Ann Arbor, MI) to assess percent body fat (%BF) using the Jackson-Pollack equation.[52] Participants then completed a football history questionnaire and baseline measures were obtained for delayed onset muscle soreness (DOMS) by placing a marked on a 10cm line anchored at each end by “no soreness” and “unbearable pain”, thirst sensation[53] (9-point scale; 1 = “not thirsty at all” 9 = “very, very thirsty”), thermal sensation (9-point scale; 0 = “unbearable cold” 8 = “unbearably hot”), level of pain (11-point scale; 0 = “no pain at all” 10 = “extremely intense pain”), fatigue (11-point scale; 0= “no fatigue at all” 10 = completely fatigued”), recovery (15-point scale; 6 = “no recovery at all” 20 = “maximum recovery”), and the 14 question Environmental Symptoms Questionnaire (ESQ) short form[54] where responses are noted on a 5-point Likert Scale ranging from 0 = ‘‘not at all,’’ to 5 = ‘‘extremely’’ to indicate the extent to which signs and symptoms are being experienced. Participants where also familiarized to the rating of perceived exertion (RPE) scale (15 point scale; 6 = “no exertion at all” 20 = “maximal exertion”)[55] that would be utilized during practice. Participants were then fitted for a heart rate (HR) monitor (Polar Model A3, Polar Electro Inc, Lake Success, NY) and the protective garment that would hold the GPS device (Catapult MinimaxX™ L4 and S4 units, Catapult Innovations, Melbourne, Australia) on the upper back between the pads and helmet. Lastly, participants were provided an ingestible core body temperature sensor (HQ Inc, Palmetto, FL) and were instructed to take the telemetric pills just prior to going to bed to ensure that the sensor was in the small intestine so that cold fluid did not alter the reading.

Testing Day Protocol:

Data collection took place during the first four days of the second week of pre-season high school football practice. The second week was selected because the full football uniform (helmet, shoulder pads, and pants equipped with thigh and knee pads) would be allowed to be worn under Arkansas state guidelines which state that full equipment can not occur until day five of pre-season.

Upon arrival for each practice, each of the participants' gastrointestinal temperature (T_{GI}) was confirmed to ensure that the pill was not passed from the previous night. Participants then provided a urine sample and body mass and pre-practice perceptual scales were obtained. (see baseline testing) Perception of thirst, thermal sensation, pain perception, level of fatigue, and level of recovery from the previous workout were recorded. Participants then filled out the environmental symptoms questionnaire (ESQ) and level of muscle soreness. The HR monitor and the Catapult vest equipped with MinimaxX™ device was activated and placed on the participant.

During practice researchers were stationed with groups of participants based on playing position. Practice schedules were provided to the researchers beforehand to ensure that the hand cooling units were available to those in the HC group. Each participant consumed fluid from their own individual water bottle which was weighed using a food scale (Salter Maxview, Taylor Precision Inc., Oak Brook, IL) to the nearest 0.01g. Exercise T_{GI} and perceptuals were recorded at each break prior to treatment or rest. Participants in both HC and CON groups were asked to remain standing for the duration of the treatment/break and were able to drink fluid ad libitum. GPS information was obtained at a frequency of 5Hz for the L3 units (n=25) and 10Hz for the S4 units (n=5) during activity and would record for the duration of the practice session. The GPS unit simultaneously recorded heart rate (HR), distance covered (DC), Player Load™(PL), meters·min^{-1}, exertion index (EI), maximum velocity (V_{max}), duration in velocity zones, and repeated high intensity exercise (RHIE) bouts throughout the session to be analyzed later using the Catapult Sprint 6.0 Software (Catapult Innovation, Melbourne, AUS). Detailed descriptions of each variable are provided in Table 2.3. Practice periods were identified using real-time data collection equipped with the 10Hz units via antenna that was set up on the sideline. Practice periods were identified and then applied to the L3 units during downloading which occurred after each practice session. Practice environmental temperature was obtained using a wet blub globe temperature device (model 4600, Kestrel Heat Stress Trackers, NK, Philadelphia, PA) during practice every 15 minutes.

At the completion of each practice participants' immediate post practice (POST) T_{GI}, RPE, thirst, thermal scales, and water bottle masses were obtained. Participants removed the GPS unit, HR monitor, provided a body mass, and urine sample, and completed the remaining perceptual scales including DOMS, pain, fatigue, and recovery levels. Participants were given another gastrointestinal temperature sensor to take for the following day's practice.

Statistical Analysis:

Demographic information are presented as (mean±SD) in Tables 2.1 and 2.2 for HS#1 and HS#2 respectively. PRE and POST data were analyzed using a 2-way repeated measures ANOVA (condition x time) analysis of variance (ANOVA). Delta (•) scores were calculated and indicate the difference between PRE and POST. When statistical significant differences were found, post-hoc *t*-tests with Bonferroni correction were used to determine differences between the conditions. Estimates of effect size were reported as partial η^2 values for ANOVA test while Cohen's *d* effect sizes were used to determine small (0.2-0.49), moderate (0.5-0.79) and large (> 0.8) effects. The maximization imputation algorithm was conducted for missing data points and were replaced only when values were missing at random based on Little's CAR. For all analyses the α level was set at 0.05. Statistical analyses were conducted using statistical software (SPSS Statistics Version 21; IBM, Inc., Chicago, IL, USA).

Data from Day 4 was included for all variables except performance data obtained via GPS. On this day less than 50% of participants were equipped with the devices because practices occurred at the same time at both schools. Therefore we have chosen not to report GPS data for both locations and these data were excluded from the analysis. Furthermore, one participant's GPS data was not utilized at HS#1 due to error, therefore the total number of participants included in the analysis where GPS was collected was n=25; HC n=12; CON n=13.

RESULTS:

High School#1: Table 2.1 contains the demographic information for the 26 subjects participating in this study. No significant differences were observed for any of the demographic variables between HC and CON. Age, body mass, height, body fat and BMI were not different (p=0.09, 0.44, 0.71, 0.20, 0.47) respectively.

High School #2: Table 2.2 contains the demographic information for the 30 subjects participating in the study from high school #2. Again no differences for the matched variables were present. Groups were similar for age, body mass, height, body fat and BMI (p=0.162, 0.824, 0.452, 0.400, 0.971) respectively.

Environmental Conditions and Practice Time

The environmental conditions including wet bulb globe temperature (WBGT) in (°C), ambient temperature (°C), and percent relative humidity (%rh) are depicted in Tables 2.4 and 2.5 for HS#1 and HS#2 respectively. The mean WBGT at HS#1 was 17.2 ± 3.0°C while at HS#2 the WBGT was 23.5 ± 3.7°C. All practices (Day 1 - Day 4) at HS#1 took place during the morning hours between 6:00am and 8:00am while all practices at HS#2 were from 6:00pm-8:00pm with the exception of Day 4 which was conducted between 6:00am-8:00am at both schools.

Body Mass Loss

High School#1: Body mass is depicted in Figure 2.1 and demonstrated a significant main effect for time, independent of group from PRE to POST on Day 1 ($F_{1,12}$= 18.511, p= 0.001, η^2= 0.607, observed power= 0.976), Day 2 ($F_{1,12}$= 9.611, p= 0.009, η^2= 0.445, observed power= 0.812), Day 3 ($F_{1,12}$= 6.953, p=

0.022, η^2= 0.367, observed power= 0.678), and Day 4 ($F_{1,12}$= 24.068, p= 0.000, η^2= 0.667, observed power= 0.994). When percent body mass loss (%BML) was calculated for all days between conditions, no significant differences were observed (t= -5.81, df=102, p=0.562) with a mean difference and 95%CI of only 0.7% (-0.3 to 0.16) between HC and CON overall. (see Figure 2.2)

High School #2: PRE and POST body masses are depicted in Figure 2.3. A significant main effect for time from PRE to POST, independent of group was observed on all days except for day 4. Significant reductions in body mass were observed on all days except day 4. Significant main effects for time, independent of group, on Day 1 ($F_{1,14}$= 23.972, p= 0.000, η^2= 0.631, observed power= 0.995), Day 2 ($F_{1,14}$= 96.83, p= 0.000, η^2= 0.874, observed power= 1.000), and Day 3 ($F_{1,14}$= 81.807, p= 0.000, η^2= 0.854, observed power= 1.000), were observed however Day 4 ($F_{1,14}$= 0.952, p= 0.346, η^2= 0.064, observed power= 0.149) demonstrated no difference from PRE to POST. %BML was not different on any day with Day 3 demonstrating the greatest difference of 0.11% (-0.54 to 0.31%) with CON demonstrating a slightly higher %BML. When all days were combined %BML was not different between HC (0.6 ± 0.6%) and CON (0.6 ± 0.6%) (t= 0.022, df= 118, p= 0.983). (see Figure 2.4)

Urine Color and Specific Gravity

High School #1: A significant main effect for time, independent of group was observed for U_{col} on Days 1, 3 and 4 (p=0.001, 0.005, and 0.002), respectively (see Figure 1.5), while U_{SG} was only significantly increased on Day 4 ($F_{1,12}$=6.848, p= 0.023, η^2=0.743, observed power=1.000). Figure 2.6 depicts the U_{col} values

PRE and POST and over the 4 days. Additionally Table 2.6 shows the means for both groups on each day as well as for all days combined.

High School #2: Means are presented for hydration status pre and post exercise for HS#2 in Table 2.7. HS#2 demonstrated significant main effects for time regardless of group for U_{col} (p=0.000, 0.001, 0.000) on Days 2, 3, and 4 respectively. A significant main effect for group, independent of time was observed for U_{col} on Day 3 ($F_{1,14}$=10.000, p=0.007, η^2= 0.417, observed power= 0.836) with CON higher than HC. (see Figure 2.7). A significant (group x time) interaction occurred on Day 1 for U_{SG} ($F_{1,14}$= 3.491, p=0.083, η^2= 0.200, observed power=0.413) with CON being MD (95%CI) 0.006 (0.002 to 0.004) higher than HC. (see Figure 2.8)

Fluid Consumption

High School #1: Fluid volume is depicted in Figure 2.9. The HC group consumed an average of 563.4 ± 147.7 mL of fluid over the four days while the CON group consumed slightly less 519.2 ± 105.8 mL. Fluid consumption each day was not significantly different between groups. Day 3 demonstrated the largest difference with HC consuming 155.51mL (-290.07 to 601.09) more than CON but this was not significantly different (t= 0.720, df= 24, p= 0.478). Only on Day 2 did CON consume more fluid (-50.08mL (-309.58 to 209.41)) than CON (t= -0.389, df= 24, p= 0.694).

High School #2: Both groups at high school #2 consumed an average of 1031.7 ± 472.4mL of fluid over the 4 days. The CON group consumed and average of 1095.1 ± 616.18mL compared to the HC group who consumed 968.42 ±

597.32mL (t= -1.143, df= 118, p= 0.255). No significant differences in fluid consumption between groups was present on any day, however Day 1 demonstrated a moderate effect ES=0.65 but no significant difference (t= -1.776, df= 28, p=0.087). Figure 1.10 depicts the fluid consumed by each group during each practice as well as the average fluid over all practice days. Average fluid intake during practice is also reported for both schools in Table 2.8.

Gastrointestinal Temperature

High School#1: Mean POST practice temperature for both groups over all practice days was 38.01 ± 0.25℃, with HC achieving a T_{GI} of 38.15 ± 0.34℃ while the CON reached 37.86 ± 0.16℃. As expected, a significant main effect of time from PRE to POST for T_{GI} was present on all practice days for all groups. No significant main effects for group, independent of time, were observed on any of the days. Post practice T_{GI} was greatest for both groups on Day 4 with T_{GI} higher in the HC group compared to CON although this was not significantly different (t= 1.817, df= 24, p=0.082), however a mean difference [HC-CON] of 0.56℃ (-0.08 to 1.20) with a moderate effect of 0.76 was observed. (see Figure 2.11)

Delta T_{GI} from PRE to POST was determined for each day as well as all days combined. No significant differences between groups were observed on any day however T_{GI} mean differences and effect sizes were 0.21℃ (-0.17 to 0.60), ES=0.20 and 0.45℃ (-0.12 to 1.02), ES= 0.64 indicating small and moderate effects for Days 1 and 4 respectively. (see Figure 2.12) When •T_{GI} for all days was pooled, HC was not different than CON (t= -1.86, df= 102, p= 0.07) but HC demonstrated slightly higher temperatures compared to CON MD= 0.22℃ (-0.02

to 0.47). When examining T_{GI} on days where T_{GI} was at its greatest (Day 1 and Day 4) unexpectedly HC trended towards being higher than CON (t= 1.978, df= 50, p=0.053) with a MD=0.33℃ (-0.01 to 0.67); ES=0.55.

Mean T_{GI} obtained during practice at each break was also calculated and again no significant differences were observed on any day. Day 2 demonstrated the greatest mean difference [HC-CON] of 0.17℃ (-0.35 to 0.69); ES= 0.26. (see Figure 1.13) PRE T_{GI}, POST T_{GI}, T_{GI} are all presented in Table 2.9.

High School #2: Mean POST practice temperature for both groups over all practice days was 37.99 ± 0.21℃ with HC obtaining a slightly lower T_{GI} (37.97± 0.25℃) compared to CON (38.01± 0.23℃). Similar to HS#1, HS#2 also demonstrated a significant main effect for time, independent of group from PRE to POST for T_{GI} on all practice days. No significant main effects for group were observed on any of the days. POST T_{GI} was greatest for CON on Day 1 (38.22 ± 0.34℃) while HC was hottest on Day 3 (38.20 ± 0.64℃). (see Figure 2.14)

Delta T_{GI} from PRE to POST was determined for each day as well as all days combined and no significant differences were observed on any day. (see Figure 2.11) Delta T_{GI} on Day 4 was greatest for HC and CON (1.37± 0.79℃, 1.56 ± 0.65℃) respectively. Although not significant (t= -0.730, df= 28, p= 0.472), the mean difference for [HC-CON] was -0.19℃ (-0.74 to 0.35); ES= 0.26 indicating a small effect in favor of the HC group. (see Figure 2.15) When T_{GI} was pooled over all practice days, HC was not different that CON (t= -0.278, df= 118, p= 0.782) but HC demonstrated slightly lower T_{GI} compared to CON MD=-0.32℃ (-0.26 to 0.196). When examining T_{GI} on days where T_{GI} was greatest

(Day 2 and Day 4), HC was slightly lower than CON MD= -0.19℃ (-0.59, 0.21) with a small effect size (ES= 0.26) but not significantly different ($t= -0.971$, $df= 58$, $p= 0.336$).

Mean T_{GI} during each break was calculated and no significant differences were observed on any day. Day 3 demonstrated the greatest mean difference for [HC-CON]= -0.27℃ (-0.59 to 0.04); ES=0.65 in favor of HC but no significant difference ($t= -1.790$, $df= 28$, $p= 0.084$). (see Figure 2.16) PRE T_{GI}, POST T_{GI}, ΔT_{GI} are all presented in Table 2.10.

Heart Rate

High School #1: Mean HR during practice for both groups was 119 ± 11bpm with the HC group demonstrating slightly higher HR (122 ± 10bpm) compared to CON (116 ± 13bpm). (see Figure 2.17) Mean heart rates during practice sessions were not different on any day indicating that both groups exercise intensity was similar, however on Day 3 mean difference [HC-CON] was 8bpm (-3 to 18); ES=0.55 with HC demonstrating a moderately higher heart rate compared to CON. (see Table 2.9)

HR_{max} obtained in during the practice sessions is depicted in Figure 2.18 as well as Table 2.9. Mean HR_{max} for both groups was 184 ± 11bpm with HC mean= 186 ± 8bpm and CON= 182 ± 13bpm over all practice sessions. HR_{max} was greatest on Day 3 for both groups however no significant differences between groups was present ($t= 1.783$, $df= 23$, $p= 0.088$) however a large effect MD= 10bpm(-2, 22): ES= 0.71 was observed with HC obtaining higher HR_{max}.

High School #2: Mean HR during practice for both groups over all practice sessions was 119 ± 10bpm with HC mean practice HR (117 ± 10bpm) slightly lower than CON (121 ± 9bpm). (see Figure 2.19). Similar to HS#1, HS#2 did not demonstrate any significant differences on any day.

During the most intense practice session on Day 2 HC obtained a HR_{max} of 182 ± 19bpm while CON was 8bpm (-10 to 25) lower however this was not found to be significant (t= 0.892, df= 28, p= 0.380). HR_{max} over all sessions is depicted in Figure 2.20 as well as Table 2.10.

Distance Covered and Meters per Minute

High School#1: The average distance covered in all 3 practices for both groups was 1975 ± 502m with HC covering almost 200m more than CON over the 3 days. (see Table 1.11) HC covered an average of 2075 ± 380m while CON covered 1883 ± 594m during each practice session. No significant differences for distance covered were observed for any practice session. On Day 3 both HC and CON covered the most distance with HC= 2926 ± 576m and CON= 2788 ± 900m however no difference was observed (t= .453, df= 23, p=0.655). (see Figure 2.21)

When examining the meters of exercise per minute of active movement, no differences between groups were observed on any day. (see Figure 2.22) Over the 3 days, the mean pace for practice in $m \cdot min^{-1}$ for all participants was 18.9 ± 4.9$m \cdot min^{-1}$. Pace for HC was 20.2 ± 4.3$m \cdot min^{-1}$ while CON was 17.7 ± 5.2$m \cdot min^{-1}$. On Day 2, HC was 3.5$m \cdot min^{-1}$ (-1.7, to 8.7), ES= 0.46 greater than CON.

High School #2: HS#2 covered 2481± 486m on average during the 3 practice sessions. (see Table 2.12) HC covered 2390 ± 526m compared to CON who covered 2572 ± 442m. (see Figure 2.23) No significant differences were observed for any practice, however a moderate ES=0.50 was observed for Day 1 with CON covering 326m (163 to 815) more distance than HC. On Day 2 both HC and CON covered the greatest distance, 2991 ± 648m and 2969 ± 541m, respectively however no significant difference was observed ($t= 0.104$, $df= 28$, $p= 0.281$).

For $m \cdot min^{-1}$ of active practice time, no differences were observed between groups on any day. (see Figure 2.24) On Day 1, HC covered $1.24 m \cdot min^{-1}$ (-4.24 to 1.24), ES= 0.53 less than CON ($t= -1.458$, $df= 28$, $p= 0.156$).

Player Load™ and Repeated High Intensity Bouts

High School#1: Player Load™ (see Table 2.3 for explanation) was determined for both groups and found that at HS#1 there were no significant differences between groups on any day. (see Figure 2.25) Both groups over all days experienced a load of 218 ± 38 with HC experiencing a load of 221 ± 23 while CON was slightly less with PL= 214 ± 48. (see Table 2.11) Both groups experienced the greatest PL on Day 3 with an average of 311 ± 57 however no significant differences were observed ($t= 0.306$, $df= 28$, $p=0.762$). The largest difference was observed on Day 2 [HC-CON]= 20 (-20, 60), ES=0.42.

The number of repeated high intensity bouts were also obtained and although no significance between groups was evident, on Day 2 HC demonstrated more high intensity bouts and a moderate difference 5 bouts (-1 to 11), ES= 0.77 was observed. (see Figure 2.26)

High School #2: PL for HS#2 was on average 272 ± 50 for both groups over all practice days. PL for HC was 267 ± 50 while CON was 276 ± 51. Although on Day 1 HC experienced the greatest difference [HC-CON] by -19 (-68 to 31) there was no significant differences on this day ($t= -0.765$, $df= 28$, $p= 0.451$) or any other day. (see Figure 2.27).

When examining the number of RHIE bouts no differences were observed between groups on any day. The average number of bouts during all practices was 47 ± 5bouts for both groups, with HC demonstrating slightly fewer bouts (45 ± 5bouts) than CON (49 ± 6bouts). (see Table 2.12) On Day 1, a moderate difference was observed between [HC-CON] of -4 (-11, 2), ES= 0.57 with CON demonstrating more bouts than HC however this was not significant ($t= -1.388$, $df= 28$, $p= 0.176$).(see Figure 2.28)

Exertion Index

High School #1: The exertion index levels for HS#1 are depicted in Figure 2.29. The average EI score for both groups was 12.5 ± 3.7 over all sessions. The average EI for HC was 12.3 ± 2.8 while CON was 11.8 ± 4.3. No significant differences were observed between groups. The greatest EI were observed on Day 3 with both groups averaging 20 ± 3 and 18 ± 7 for HC and CON respectively [HC-CON] MD= 2 (3 to 6), ES= 0.37. (see Table 2.11)

High School #2: EI for HS #2 is depicted in Figure 1.30. EI was not significant between conditions on any day. The average EI over the 3 days for both groups was 15.3 ± 2.9 with HC= 15.4 ± 2.9 and CON= 15.2 ± 3.0. On Day 2 the players experienced the greatest EI in both groups (17.9 ± 4.2) however the MD for [HC-CON] was only 1.0 (-2.0, 4.0).

Max Velocity and Distance Covered in Velocity Zones

High School #1: There were no differences for maximum velocity (V_{max}) between groups on any day. Average V_{max} for both groups over all practice sessions was 14.6 ± 1.6mi·h^{-1} with V_{max} for HC and CON being (14.9 ± 1.4mi·h^{-1} and 14.4 ± 1.8mi·h^{-1}) respectively. V_{max} in each session for both groups are depicted in Figure 2.31.

When examining the distance covered in the 7 velocity zones ranging from walking to maximal sprinting between HC and CON on Days 1-4 there were no significant differences in the distance covered in any of the speed zones. On Day 2 in zone 2 (4.5-6.5mi·h^{-1}); HC appeared to cover more distance than CON MD= 176m (-58 to 410), ES= 0.62 although this was not significant (t= 1.552, df= 23, p=0.134). Furthermore, on Day 1 in zone 3 (6.5-8.5mi·h^{-1}) HC covered 35m (-15, 85), ES= 0.57 more than CON. Distance covered in each velocity zone is represented in Figure 2.32.

High School #2: V_{max} was not different between groups during any practice session. The average velocity for both groups was 15.1 ± 2.1mi·h^{-1}. HC average V_{max} was slightly higher than CON during practice sessions (15.2 ± 2.3mi·h^{-1} and 14.9 ± 2.0mi·h^{-1}) respectively. V_{max} in each session for both groups are depicted in Figure 1.33 as well as Table 2.12.

Distance covered in each velocity zone revealed moderate differences in zone 2 on Day 1, zones 4 (8.5-10.5mi·h^{-1}) and 7 (>15mi·h^{-1}) on Day 3. Mean difference [HC-CON] for zone 2 on Day 1 and zone 7 on Day 3 revealed that HC covered -217m (-444 to 10), ES= 0.72 and -10m (-25, 4), ES= 0.57 less than CON. Zone 4 on Day 3 demonstrated the opposite finding with HC covering 53m (-26 to 133), ES= 0.50. Effect sizes and distances in velocity zones are depicted in Figure 2.34.

PRE and POST Perceptual Measures: Thirst, Thermal, ESQ, Pain, Fatigue, Recovery, DOMS

High School#1: Independent of time a significant main effect for group was observed for the perception of thirst on Day 2 ($F_{1,12}$= 5.270, p= 0.041, η^2= 0.305, observed power= 0.560) however no other significant main effects or interaction were observed on any other days for thirst. (see Figure 2.35) Thermal perception however, did experience significant main effects for time independent of group, on Days 2 and 3 ($F_{1,12}$= 13.479, p= 0.003, η^2= 0.529, observed power= 0.920; $F_{1,12}$= 5.658, p= 0.035, η^2= 0.320, observed power= 0.589) respectively. On both Day 2 and 3 thermal sensation increased regardless of group. (see Figure 2.36) ESQ was extremely low relative to other studies and the average for either group never exceeded 10 out of a possible 60. However, even in these relatively cool conditions, significant main effects for time were observed on all days with POST values being consistently higher than PRE for both groups. On two occasions, Days 1 and 2, significant main effects for group were observed Day 1: $F_{1,12}$= 9.914, p= 0.008, η^2= 0.452, observed power= 0.824 and Day 2: $F_{1,12}$= 12.916, p=

0.004, η^2= 0.518, observed power= 0.909. On both days, CON experienced higher levels on the ESQ compared to HC as depicted in Figure 2.37. Pain and recovery levels both demonstrated significant main effects for time on all days as depicted in Figures 2.38 and 2.39, however no between group or significant interactions were observed. As expected pain was slightly but significantly elevated immediately POST compared to PRE. Level of fatigue from PRE to POST demonstrated a main effect for time on Days 2-4 with fatigue levels increasing POST in all groups. (see Figure 2.40) Lastly, a significant main effect for time was observed for DOMS on all days with POST practice levels being higher than PRE for both groups.(see Figure 2.41) All perceptual data for PRE and POST activity are presented in Table 1.13.

High School #2: Unlike HS#1, no main effect for group was observed for the perception of thirst on any day, however significant main effects for time were observed on all days with POST practice thirst levels being significantly increased compared to PRE in both groups. (see Figure 2.42) Thermal perception for HS#2 was very interesting because on Day 3 a significant interaction (group x time) occurred ($F_{1,14}$= 5.508, p= 0.034, η^2= 0.282, observed power= 0.589). At POST HC was significantly different from CON (p= 0.024) with HC demonstrating a higher thermal perception than CON. On the following day, Day 4 a significant main effect for group was observed ($F_{1,14}$= 6.087, p= 0.027, η^2= 0.282, observed power= 0.632) and again HC had increased thermal perception than CON. On all days except Day 1, a significant main effect for time was observed for thermal sensation as depicted in Figure 2.43. ESQ, pain, recovery, fatigue and DOMS all had significant main effects for time from PRE to POST independent of group. (see Figures 2.44-2.48) Although relatively low on the pain scale spectrum, on Day 2 pain demonstrated a significant main effect for group ($F_{1,14}$= 5.249, p= 0.038, η^2= 0.273, observed power= 0.569) as well as a significant group x time interaction ($F_{1,14}$= 10.475, p= 0.006, η^2= 0.428, observed power= 0.853) with CON experiencing more pain than HC POST. DOMS on Day 2 also showed a significant interaction ($F_{1,14}$= 5.52, p= 0.034, η^2= 0.283, observed power= 0.590). Between group t-tests however did not show that HC experienced significantly less DOMS than CON (p=0.088), but the MD was -10.7 (-23.2 to 1.70). All perceptual data for PRE and POST activity are presented in Table 2.14.

In-Practice Perceptual Measures: RPE, Thirst, Thermal

High School#1*:* Rating of perceived exertion (RPE), thirst sensation and thermal perception were asked to each player during their breaks after the athlete had started cooling or consuming fluids. At no time were there differences between groups for RPE, thirst of thermal. Only on Day 4 was thermal perception slightly lower in the HC group compared to CON MD= -2 (-5 to 1) with a moderate effect size of 0.69. Players in both groups consistently indicated they were working “somewhat hard” to “hard” during all practice sessions while thirst was between “a little thirsty” and “moderately thirsty” and thermal was between “comfortable” and “warm”. (see Figures 2.49-2.51) All perceptual measures obtained during practice are presented in Table 2.15.

High School #2: RPE, thirst and thermal were also not significantly different during breaks at HS#2 although on Day 1 RPE was nearly different than CON ($p= 0.051$). MD for [HC-CON] was -1 (-3, 0), ES= 0.74. Players in both groups indicated that they were working “somewhat hard” to “hard” during most of the practice sessions, while thirst and thermal were consistently surrounding “moderately thirsty” and “warm” respectively. (see Figures 2.52-2.54 and Table 2.16)

Sleep

High School#1: Total sleep hours and number of nap hours were not different between groups throughout the four days of data collection. (see Figure 2.55) Furthermore, when asked their level of sleep quality as well as level of rest, again no differences were observed between groups on any day. (see Figure 2.56-2.57) This indicates that both groups were similar outside of the practice sessions.

High School #2: Total sleep hours and nap hours at HS#2 were not different between groups over all days of data collection. (see Figure 2.58) Sleep quality was also similar between groups on all days. (see Figure 2.59) Rest on Day 1 was lower but only varied slightly [HC-CON]= -1, (-1 to 0); $p= 0.005$. All other days, level of rest was similar between groups. (see Figure 2.60)

DISCUSSION:

The ability to remove heat from the body during breaks in American football is critical to athletic trainers, coaches and healthcare providers. Football has been well documented to experience a large amount of heat-related illness and deaths each year.[22,23,56] With numerous modalities on the consumer market designed to reduce body temperature during exercise, it is critical that an effective device be utilized for best practice when implementing heat-illness prevention strategies. Through our observational field study of peripheral hand cooling on physiological, perceptual, and performance variables during pre-season high school football, we quantified the effect of peripheral hand cooling with negative pressure. This investigation was the first time that a peripheral hand cooling with negative pressure was examined in high school football players in the field setting. Additionally, this was the first time that GPS and accelerometry data with tri-axial accelerometers, gyroscope and magnetometer, was utilized to document performance during pre-season football over four successive days of pre-season practice in August.

Based on our findings, we are unable to conclude that the use of peripheral hand cooling during breaks in high school football practice was effective in mildly hyperthermic individuals. Based on our findings, the participants at both schools did not experience any reduction in T_{GI} during or POST practice. Furthermore, the •T_{GI} was not different between the HC and CON groups on any of the days at either of the high schools. In addition we observed small to moderate effects for perceptual and performance measures. We feel that the there are both informative and positive findings that can be taken from this investigation that add to the body of knowledge that will enhance future investigations in this area.

Discussion of findings related to T_{GI}:

This investigation forms a better understanding of hand cooling with negative pressure under these specific environmental conditions. Although peripheral hand cooling with negative pressure demonstrated no statistically significant differences in physiological measures of T_{GI}, a moderate increase in the CON group was observed (ES= 0.65) in HS#2. Interestingly, this corresponds with the highest ambient temperature observed out of all four days of data collection at this school (28.1°C± 2.6) and may suggest that with higher environmental temperature larger effects may be evident. Other research in the area of intermittent cooling during exercise using other modalities over other regions of the body such as ice vest or combined head and neck cooling have observed more effective reductions in body temperature during hyperthermic exercise[57,58] while others have observed little change[36,37,59–61] Kenny et al.[57] observed that during uncompensable heat stress conditions while wearing chemical protective gear that esophageal temperature was decreased by 0.29°C and time to exhaustion was increased by approximately 12% or 12 minutes when wearing the vest compared to the condition without the ice vest. Ansley et al.[58] also observed the influence of cooling during endurance cycling by cooling the head and observed a 51% increase in performance and reduced tympanic temperature. While both of these studies differ in terms of type of exercise and environment, important conclusions can be gathered in terms of the type of stress that was placed on these subjects. One rationale as to why we were unable to observe changes in body temperature in the current study was that participants did not experience the same level of uncompensable heat stress that is normally accustomed to Arkansas in the second week of August. The national weather service climatology reports ranked August 2013 as the 20th coldest (mean= 75.4°F) since 1950.[62] Normal average daily high temperatures from 1981-2010 for the same four days are 32.0°C, [90.0°F] however the average during this study was 25.6°C, [78.0°F]. With this in mind, similar to the study conducted by Ansley et al.[58] and unlike the study conducted by Kenny et al.,[57] uncompensable heat stress was not obtained in our investigation. Research with similar findings demonstrate little to no effect of cooling has been documented in three separate studies,[60,61,63] one review examining a neck cooling collar,[59] and two studies[36,37] using ice vests. Our study as well as the aforementioned studies all demonstrated little to no effect on body temperature, however when taken into context with the study by Kenny et al.,[57] we may begin to make logical comparisons and develop a potential rationale as to why this occurred.

One potential reason for the lack of cooling effectiveness observed in the current and other studies that failed to show reductions in body temperature was demonstrated in 1971 by Nunneley et al.[64] They investigated the effect of head cooling using a water-perfused cap in three different environmental temperatures (20.0, 30.0 and 40.0°C). In that study the cooling cap was only effective in the 40.0°C condition when the thermal stress was at its highest while no effect was observed in the 20.0 and 30.0°C trials. These results suggest that only in the presence of a larger thermal stress, does the cooling cap demonstrate effectiveness. So in cases where the thermal stress is inadequate (i.e. the present study), significant thermal reduction may not be observed or be underreported. This same thought has been echoed by Tyler et al.[59] who suggest that had the heat stress been greater when T_{GI} reaches higher levels, similar to the UCHS experienced in the study by Kenny et al.[57] There is a possibility that the peripheral hand cooling device may have demonstrated a larger effect and therefore we were unable to accurately quantify its effect, however we did observe moderate effects in the multitude of variables examined in this investigation.

Potential mechanisms related to changes in HR:

Heart rate demonstrated some interesting changes between groups in one of the schools tested. On Day 3 in HS#1 both average HR and HR_{max} during practice were moderately elevated (ES= 0.55) and (ES= 0.71) respectively, in the HC group. Although some may view this increase in HR as a negative physiological outcome, it is possible that participants were able to exercise at a higher intensity due to the suppression of pain and fatigue as proposed by Burke et al.[65] who theorize that increased pain receptor thresholds and decreased perception of contraction intensity may be experienced. Although not directly related, this conclusion is eerily similar to other work in the area of arthrogenic muscle inhibition and the use of cryotherapy as a disinhibitory modality.[66–68] Cryotherapy over the knee joint has demonstrated increases in quadriceps activation ratios through disinhibition of sensory information to higher motor control centers in the bran. The effect of the hand cooling device on 1a afferents may be operating along the same mechanistic pathway inhibiting muscular fatigue enabling for increased muscle activation and increased heart rate to meet the demands of the exercising muscle as suggested by Kwon et al.[69] This theory is not well understood in the context of hand cooling and deserves further exploration.

Other research in the context of cooling has observed similar outcomes indicating no change in HR[58,59,61] and only one study observed a significant increase in HR.[63] Anecdotally, research from our lab using the same modality determined that HR was slightly (ES= 0.28 to 0.40) reduced for 30 minutes during exercise following intermittent hand cooling with fluid ingestion compared to control. Furthermore cooling and fluid replacement demonstrated an average of 8% improvement following a performance battery consisting of sprinting, agility, and balance tests suggesting the reduced cardiovascular drift from the hydration protocol may have reduced cardiovascular stress. This reduction in cardiovascular stress may have allowed for increased cardiac output when the demand was needed for the performance battery.

Mechanisms behind changes in perceptual measures:

Further examination of POST and average RPE in HS#1 revealed moderate effects on Day 1 (ES= 0.74) and Day 4 (ES= 0.69) in the CON group even though heart rate, total distance, Player Load™ and number of RHIE bouts were similar between groups. These results suggest that the cooling device may be reducing one's RPE which is similar to the findings of Kwon et al.[70] who found RPE to be reduced during palm cooling between bench press repetitions.

Reduction of RPE has been proposed to be reduced via 1 of 2 mechanisms. The first mechanism is the "Gate Control Theory" by Melzak and Wall[71] where application of cold results in the closing of the gate (substantia gelatinosa) and blockade of sensory thermal information to the spinal cord, thus reducing perception. The other theory is through the temporary override of fatigue mechanisms as proposed in the "Central Fatigue Theory" by Noakes et al.,[72] which describes the central processing of peripheral information and the control of fatigue by the brain. Based on this proposed theory, the brain decreases neuromuscular stimulus in response to afferent sensory information and decreases the activation of motor units resulting in fatigue. It has been suggested that application of cold may override this fatigue mechanism resulting in reduced RPE as well as other measures such as thermal sensation and pain. With that being said, thermal sensation (see Table 2.14) on Day 3 in HS#2 was significantly lower (p=0.024) POST for the CON group however •T_{GI} was slightly less in the CON condition on that day. Keeping in mind the proposed mechanisms for alteration in pain sensation previously described, the CON group in HS#2 on Day 2 reported a significantly higher (p= 0.004) pain level than HC however the mean difference in pain level was very small 2 ± 1 "mild" to "moderate" pain level described above HC as indicated in Figure 2.45. This increased level of pain was slightly higher for the CON group when compared to values indicated on the other three days, but not significantly different. Also of note, DOMS was higher in the CON group and although not significant (p=0.08), demonstrated a moderate effect (ES= 0.65), which may explain the increase in pain on that day. Future research should examine peripheral cooling on RPE, pain, fatigue and other perceptual measures that may indicate fatigue and exercise performance. Additionally, if less exertion is perceived as a result of decreased fatigue, might reductions in these perceptual measures be separate from modalities whose goal is to reduce body temperature?

Discussion of findings related to performance measures:

The validity and reliability of the Catapult MinimaxX™ devices has been documented in the literature when examining total distance covered (percentage typical error of measurement [%TEM] < 5%), peak speed (%TEM, 5-10%) and Player Load™ (%TEM, 4.9%).[73] Within-device reliability has also been validated in the laboratory setting (CV= 1.04%) and field setting (CV= 1.9%).[74] Compared to 5Hz devices, 10Hz GPS devices have demonstrated to be 2-3x more accurate and 6-fold more reliable when measuring instantaneous velocity.[75] In the current study, a majority of the devices 25 out of 30, were 5Hz while the remaining 5 were 10Hz; therefore, measures of instantaneous velocity may be less accurately depicted. The tackle detection function has reported mixed results when examining various sports such as Australian football,[76] cricket, and other field based sports[77] and was not measured in the current study based on this information. Furthermore it was not the primary goal of this investigation to examine player statistics related to the number of tackles in practice.

In our examination distance covered, exertion index, and maximum velocity demonstrated little difference between groups. Although it was hypothesized that there may be modifications as a result of the cooling intervention, both locations had well designed breaks built into their practice schedules to ensure the work to rest ratios were adequate for hydration and rest between drills. Given these well-designed practice schedules, it was not surprising that we did not see any changes in these measures. However, close examination of the bouts of repeated high intensity efforts and distance covered in specific velocity zones achieved by the players, revealed some moderate effects. In HS#1 on Day 2 CON had moderately less number (ES= -0.77) of RHIE bouts as well as moderately less distance covered in velocity zone 2 (ES= -0.62) and zone 3 (ES= -0.57) compared to HC. (see Figure 2.32) At HS#2 on Day 1 number of RHIE bouts were higher in the CON (ES= 0.57) as depicted in Figure 2.28 and moderately increased for distance covered in zone 1 (ES= 0.72) and zone 7 (ES= 0.57). Distance covered in Zone 4 however, was increased in the HC group on Day 3, which demonstrated the greatest WBGT temperature of all days at HS#2 (WBGT= 25.4℃ ± 1.4). Future research should continue to examine the velocity zones during pre-determined football specific drills to better examine the effectiveness of the cooling between drills. This may more precisely elucidate the number of RHIE bouts and distance covered in velocity zones thus providing more accurate assessment of performance.

Limitations

The current field study is not without limitations. We feel we have adequately described the decreased environmental conditions in the discussion and the accuracy of the 5Hz GPS velocity data, however we have yet to mention the potential for inaccurate readings of the telemetric pill. It is possible that not all athletes ingested the pill set forth in the protocol the night before activity. On a few occasions, athletes at both schools reported to the testing day having passed the pill from the night before. Unfortunately, we are unable to determine the individual rates in which athletes pass the sensors, and administration of a pill in the hours preceding a practice would have been unreliable. This is a real-world scenario and medical staff will be pressed with the same issues in the field when attempting to monitor their athletes for safety. Another limitation of the current study design was that we were unable to pool the data from both teams because of the different practice intensities, environmental conditions (morning vs. evening), and break schedules. Had we been able to dictate these variables, we might have been able to compare between groups on both teams. Another notable limitation was that players consumed a variety of fluids ranging from carbohydrate electrolyte beverages to water during practice and we are unable to account for the performance benefits associated with different beverages. Last, we did not control or monitor the nutritional intake of the players in either group, which may have been beneficial to their performance and recovery. Future research in field settings should attempt to control these variables without altering the normal intake and hydration habits outside of football practice in an effort to decrease the variability.

CONCLUSION:

Peripheral cooling of the hand with negative pressure demonstrated mixed effectiveness during pre-season high school football practice in matched players on two teams. We are unable to conclude that hand cooling intermittently during regularly scheduled breaks was effective in the reduction of physiological measures (T_{GI} and HR), perceptual measures (RPE, thermal sensation, fatigue, pain, and DOMS) or performance measures (distance covered, RHIE bouts, velocity zones, and exertion index) although moderate effects were observed in multiple areas between groups. Strategies to prevent body temperature from reaching dangerous levels of hyperthermia (> 40°C,[104°F]) are very much encouraged when other less convenient methods of rapid cooling are not present. This is especially true for the sport of American football where we have seen a number of heat-related deaths. Coaches, researchers and members of the sports medicine team should continue to test the effectiveness hand cooling in the field under more strenuous conditions where heat stress is uncompensable.

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Tables:

Table 2.1

High School #1: Demographics

	n	Age (y)	Height (m)	Body Mass (kg)	Body Mass Index	% Body Fat
HC	13	16±1	1.8±0.1	90.1±20.2	25.5±9.3	19.4±9.8
CON	13	16±1	1.8±0.1	85.1±17.3	27.4±5.3	18.6±10.1

Table 2.2

High School #2 : Demographics

	n	Age (y)	Height (m)	Body Mass (kg)	Body Mass Index	% Body Fat
HC	15	17±1	1.8±0.1	88.9±15.1	27.2±4.4	24.1±9.1
CON	15	16±1	1.8±0.1	90.1±14.8	27.2±4.8	21.4±8.3

Table 2.3

Description of Variables Utilized To Quantify Performance Using the Catapult Software	
Variable	**Description**
Exertion Index (EI)	A parameter used to describe the physical load and is based on the sum a weighted accumulation of speed over 60 seconds thus ensuring that a sharp effort and a long duration effort are quantified equally.
Repeated High Intensity Effort Bouts (RHIE)	Composed of the number of repeated high intensity effort bouts that the athlete experiences. A RHIE is when an athlete expeiences >3 qualifying high intensity efforts such as a tackle, a sprint, or acceleration in any combination all within 21 seconds.
Player Load (PL)	Is an instantaneous rate of change of acceleration that is scaled by a factor of a 1000. And accumulates with tackles, and other non-running activities.

Table 2.4

High School #1: Environmental Conditions

	Day 1	Day 2	Day 3	Day 4	Average
WBGT (°C)	23.0±0.6	17.6±0.7	14.5±1.2	16.2±1.2	17.2±3.0
Ambient Temp (°C)	24.8±0.4	18.3±0.7	15.6±0.8	17.8±0.7	18.5±3.2
Humidity (%rh)	95.7±3.5	89.3±4.2	81.0±4.3	72.9±3.7	83.9±9.0

Table 2.5

High School #2: Environmental Conditions

	Day 1	Day 2	Day 3	Day 4	Average
WBGT (°C)	24.0±0.2	25.0±2.9	25.4±1.4	15.9±0.5	23.5±3.7
Ambient Temp (°C)	24.8±0.3	28.1±2.6	26.8±1.5	17.1±0.7	25.5±4.2
Humidity (%rh)	90.6±2.4	38.6±6.8	36.2±4.7	82.5±4.1	56.8±25.4

Table 2.6

High School # 1: Hydration Status PRE & POST Exercise

	Condition	U_{COL} PRE	POST	U_{SG} PRE	POST
Day 1	HC	3 ± 1	5 ± 2	1.023 ± 0.004	1.023 ± 0.008
	CON	3 ± 1	5 ± 1	1.019 ± 0.008	1.022 ± 0.008
Day 2	HC	3 ± 1	3 ± 2	1.022 ± 0.005	1.019 ± 0.010
	CON	3 ± 2	3 ± 1	1.021 ± 0.006	1.021 ± 0.008
Day 3	HC	3 ± 1	4 ± 2	1.021 ± 0.005	1.021 ± 0.007
	CON	3 ± 1	5 ± 2	1.020 ± 0.006	1.021 ± 0.006
Day 4	HC	4 ±1	5 ± 1	1.020 ± 0.005	1.022 ± 0.006
	CON	4 ± 1	5 ± 1	1.021 ± 0.005	1.023 ± 0.006
All Days	HC	3 ± 1	4 ± 1	1.022 ± 0.003	1.021 ± 0.006
	CON	3 ± 1	4 ± 1	1.020 ± 0.005	1.022 ± 0.006

Table 2.7

High School # 2: Hydration Status PRE & POST Exercise

	Condition	U_{COL} PRE	POST	U_{SG} PRE	POST
Day 1	HC	3 ± 1	3 ± 2	1.023 ± 0.007	1.022 ± 0.008
	CON	3 ± 1	4 ± 2	1.024 ± 0.010	1.028 ± 0.007
Day 2	HC	3 ± 2	4 ± 1	1.018 ± 0.010	1.022 ± 0.008
	CON	3 ± 1	4 ± 2	1.020 ± 0.007	1.025 ± 0.006
Day 3	HC	2 ± 2	4 ± 1	1.016 ± 0.009	1.022 ± 0.006
	CON	4 ± 2	5 ± 1	1.020 ± 0.009	1.026 ± 0.004
Day 4	HC	4 ± 1	5 ± 1	1.025 ± 0.005	1.026 ± 0.005
	CON	4 ± 1	5 ± 2	1.024 ± 0.008	1.027 ± 0.006
All Days	HC	3 ± 1	4 ± 1	1.021 ± 0.007	1.023 ± 0.007
	CON	3 ± 1	5 ± 2	1.022 ± 0.009	1.026 ± 0.006

Table 2.8

Fluid Volume Consumed During Football Practice at Both Schools

	Condition	High School # 1 Fluid Volume (mL)	High School # 2 Fluid Volume (mL)
Day 1	HC	645.0 ± 324.7	1018.7 ± 472.3
	CON	591.3 ± 354.9	1299.8 ± 386.9
Day 2	HC	445.9 ± 309.0	1479.5 ± 638.8
	CON	546.0 ± 331.7	1548.3 ± 517.8
Day 3	HC	748.6 ± 606.0	1012.6 ± 501.3
	CON	593.1 ± 488.6	1006.9 ± 396.4
Day 4	HC	363.8 ± 300.1	447.8 ± 180.8
	CON	346.5 ± 231.7	467.6 ± 221.1
All Days	HC	563.4 ± 249.9	989.6 ± 253.6
	CON	519.2 ± 221.0	1023.0 ± 260.8

Table 2.9

High School # 1: Physiological Measurements of Body Temperature and Heart Rate

	Condition	Post T_{GI} (°C)	T_{GI} (°C)	ΔT_{GI} (°C)	HR_{max} (bpm)	HR (bpm)
Day 1	HC	38.02 ± 0.47	38.02 ± 0.23	1.09 ± 0.52	180 ± 15	122 ± 14
	CON	37.77 ± 0.39	38.03 ± 0.47	0.88 ± 0.47	183 ± 18	118 ± 14
Day 2	HC	37.94 ± 0.50	38.03 ± 0.51	1.02 ± 0.48	184 ± 14	123 ± 11
	CON	37.75 ± 0.49	37.86 ± 0.76	0.91 ± 0.62	179 ± 17	118 ± 15
Day 3	HC	37.98 ± 0.49	37.89 ± 0.53	1.12 ± 0.57	194 ± 13	120 ± 8
	CON	37.83 ± 0.90	37.78 ± 0.63	1.00 ± 0.90	184 ± 15	113 ± 15
Day 4	HC	38.66 ± 0.66	38.43 ± 0.53	1.45 ± 0.67	--	--
	CON	38.10 ± 0.89	38.35 ± 0.65	1.00 ± 0.74	--	--
All Days	HC	38.15 ± 0.34	38.09 ± 0.45	1.17 ± 0.56	186 ± 8	122 ± 10
	CON	37.86 ± 0.16	37.99 ± 0.63	0.95 ± 0.67	182 ± 13	116 ± 13

Table 2.10

High School # 2: Physiological Measurements of Body Temperature and Heart Rate

	Condition	Post T_{GI} (°C)	T_{GI} (°C)	ΔT_{GI} (°C)	HR_{max} (bpm)	HR (bpm)
Day 1	HC	38.10 ± 0.33	37.84 ± 0.39	0.99 ± 0.46	177 ± 21	104 ± 12
	CON	38.22 ± 0.34	38.06 ± 0.38	0.92 ± 0.78	181 ± 19	109 ± 10
Day 2	HC	37.67 ± 0.48	37.75 ± 0.48	0.57 ± 0.63	182 ± 19	131 ± 9
	CON	37.88 ± 0.33	37.84 ± 0.40	0.76 ± 0.52	174 ± 27	130 ± 15
Day 3	HC	38.20 ± 0.64	37.84 ± 0.47	0.93 ± 0.61	164 ± 21	117 ± 21
	CON	38.18 ± 0.27	38.12 ± 0.35	0.74 ± 0.29	167 ± 20	124 ± 14
Day 4	HC	37.92 ± 1.24	37.85 ± 1.08	1.37 ± 0.79	--	--
	CON	37.75 ± 0.92	37.66 ± 0.90	1.56 ± 0.65	--	--
All Days	HC	37.97 ± 0.25	37.82 ± 0.60	0.96 ± 0.62	174 ± 14	117 ± 10
	CON	38.01 ± 0.23	37.92 ± 0.51	1.00 ± 0.46	174 ± 12	121 ± 9

Table 2.11

High School # 1: Performance Measures Obtained via GPS

	Condition	Day 1	Day 2	Day 3	All Days
Distance (m)	HC	1169 ± 390	2128 ± 538	2927 ± 577	2075 ± 380
	CON	1107 ± 422	1753 ± 675	2789 ± 900	1883 ± 594
$m \cdot min^{-1}$	HC	19.3 ± 6.8	22.0 ± 5.5	19.4 ± 3.7	20.2 ± 4.3
	CON	16.9 ± 5.4	18.5 ± 6.9	17.7 ± 6.0	17.7 ± 5.2
Player Load	HC	139 ± 32	211 ± 37	315 ± 39	221 ± 23
	CON	144 ± 32	191 ± 56	308 ± 72	214 ± 48
Number of RHIE Bouts	HC	19 ± 5	35 ± 6	49 ± 8	34 ± 5
	CON	18 ± 5	30 ± 7	49 ± 11	33 ± 6
Exertion Index	HC	6 ± 3	14 ± 4	20 ± 3	13 ± 3
	CON	5 ± 3	12 ± 5	18 ± 7	12 ± 4
Max Velocity ($m \cdot s^{-1}$)	HC	13.8 ± 1.8	15.2 ± 1.6	15.7 ± 1.5	14.9 ± 1.4
	CON	13.7 ± 1.9	14.2 ± 2.2	15.0 ± 1.8	14.4 ± 1.8

Table 2.12

High School # 2: Performance Measures Obtained via GPS

	Condition	Day 1	Day 2	Day 3	All Days
Distance (m)	HC	2308 ± 759	2991 ± 648	1871 ± 526	2390 ± 526
	CON	2634 ± 528	2969 ± 541	2113 ± 455	2572 ± 442
$m \cdot min^{-1}$	HC	11.8 ± 3.1	21.6 ± 4.7	19.3 ± 3.2	17.5 ± 3.0
	CON	13.3 ± 2.9	21.4 ± 3.9	19.7 ± 4.2	18.1 ± 3.2
Player Load	HC	289 ± 79	310 ± 53	202 ± 55	267 ± 50
	CON	308 ± 51	308 ± 74	212 ± 42	276 ± 51
Number of RHIE Bouts	HC	47 ± 10	50 ± 6	39 ± 10	45 ± 4
	CON	51 ± 7	53 ± 9	42 ± 6	49 ± 6
Exertion Index	HC	16 ± 3	18 ± 5	12 ± 2	15 ± 3
	CON	16 ± 4	17 ± 3	12 ± 3	15 ± 3
Max Velocity ($m \cdot s^{-1}$)	HC	15.5 ± 5.2	15.7 ± 1.6	14.3 ± 1.6	15.2 ± 2.3
	CON	14.1 ± 2.8	15.7 ± 1.4	15.0 ± 3.0	14.9 ± 2.0

Table 2.13

High School # 1: Perceptual Measures Before and After Activity

	Condition	Thirst		Thermal		Pain		Recovery		Fatigue		ESQ		DOMS	
		PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Day 1	HC	3 ± 1	3 ± 2	5 ± 1	4 ± 1	1 ± 1	1 ± 2	15 ± 3	12 ± 3	2 ± 2	3 ± 2	3 ± 3	5 ± 2	10 ± 9	24 ± 22
	CON	3 ± 2	4 ± 2	4 ± 1	5 ± 1	1 ± 1	2 ± 1	15 ± 2	13 ± 3	3 ± 2	4 ± 2	4 ± 3	8 ± 5	17 ± 15	33 ± 22
Day 2	HC	3 ± 1	3 ± 1	4 ± 1	5 ± 1	1 ± 1	2 ± 2	15 ± 3	13 ± 3	2 ± 2	3 ± 2	3 ± 2	5 ± 2	14 ± 14	28 ± 25
	CON	4 ± 2	4 ± 2	4 ± 1	5 ± 1	1 ± 1	3 ± 2	14 ± 2	13 ± 3	3 ± 2	4 ± 2	5 ± 3	7 ± 4	19 ± 15	33 ± 22
Day 3	HC	3 ± 1	3 ± 1	4 ± 1	4 ± 1	1 ± 1	2 ± 2	17 ± 2	13 ± 4	2 ± 3	3 ± 2	3 ± 2	7 ± 4	16 ± 14	32 ± 29
	CON	3 ± 1	3 ± 2	4 ± 1	4 ± 1	1 ± 1	2 ± 2	15 ± 2	13 ± 3	2 ± 2	3 ± 2	4 ± 1	6 ± 4	18 ± 17	29 ± 23
Day 4	HC	3 ± 1	3 ± 2	3 ± 1	4 ± 1	1 ± 1	4 ± 3	15 ± 1	12 ± 2	2 ± 1	3 ± 3	4 ± 4	7 ± 4	20 ± 17	46 ± 27
	CON	3 ± 2	4 ± 1	4 ± 1	4 ± 1	1 ± 1	3 ± 2	16 ± 2	13 ± 2	2 ± 1	3 ± 2	3 ± 2	6 ± 4	15 ± 14	34 ± 21
All Days	HC	3 ± 1	3 ± 1	4 ± 1	4 ± 1	1 ± 1	2 ± 2	16 ± 2	12 ± 3	2 ± 2	3 ± 2	4 ± 2	6 ± 3	15 ± 12	33 ± 22
	CON	3 ± 1	4 ± 1	4 ± 1	4 ± 1	1 ± 1	2 ± 2	15 ± 2	13 ± 2	2 ± 2	4 ± 2	4 ± 1	7 ± 3	17 ± 14	32 ± 20

Table 2.14

High School # 2: Perceptual Measures Before and After Activity

	Condition	Thirst		Thermal		Pain		Recovery		Fatigue		ESQ		DOMS	
		PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Day 1	HC	3 ± 1	4 ± 1	5 ± 1	5 ± 1	1 ± 1	2 ± 1	14 ± 2	12 ± 3	2 ± 1	4 ± 2	5 ± 3	9 ± 6 *	21 ± 12	30 ± 13
	CON	3 ± 1	4 ± 1	5 ± 1	5 ± 1	1 ± 1	2 ± 1	15 ± 2	13 ± 2	2 ± 2	4 ± 1	4 ± 3	8 ± 3	20 ± 12	30 ± 10
Day 2	HC	3 ± 1	4 ± 2	4 ± 1	5 ± 1	1 ± 1	1 ± 1	14 ± 2	13 ± 2	2 ± 1	4 ± 1	4 ± 3	6 ± 5 *	22 ± 12	24 ± 13
	CON	3 ± 1	4 ± 2	4 ± 1	5 ± 1	1 ± 1	3 ± 1 *	15 ± 2	13 ± 1	2 ± 2	3 ± 2	4 ± 3	7 ± 4	19 ± 15	35 ± 20
Day 3	HC	2 ± 1	5 ± 2	4 ± 0	6 ± 1 *	1 ± 1	1 ± 1	15 ± 2	13 ± 2	1 ± 1	4 ± 2	3 ± 3	7 ± 4	16 ± 9	28 ± 18
	CON	2 ± 1	5 ± 2	4 ± 0	5 ± 1	1 ± 1	2 ± 1	14 ± 2	13 ± 2	2 ± 2	4 ± 2	5 ± 3	7 ± 3	20 ± 20	35 ± 21
Day 4	HC	4 ± 2	4 ± 2	4 ± 1	5 ± 1	1 ± 1	2 ± 1	13 ± 2	12 ± 1	2 ± 2	4 ± 1	6 ± 4	9 ± 6	18 ± 8	28 ± 11
	CON	3 ± 1	4 ± 2	4 ± 1	5 ± 1	1 ± 1	2 ± 1	14 ± 2	12 ± 2	2 ± 1	3 ± 1	5 ± 3	7 ± 3	20 ± 17	34 ± 17
All Days	HC	3 ± 1	4 ± 2	4 ± 1	5 ± 1	1 ± 1	1 ± 1	14 ± 2	13 ± 2	2 ± 1	3 ± 2	5 ± 3	8 ± 5	19 ± 10	28 ± 14
	CON	3 ± 1	4 ± 1	4 ± 1	5 ± 1	1 ± 1	2 ± 1	15 ± 2	13 ± 2	2 ± 2	3 ± 2	5 ± 3	8 ± 3	20 ± 16	33 ± 17

Table 2.15

High School # 1: Perceptual Measures During Activity

	Condition	RPE	Thirst	Thermal
Day 1	HC	14 ± 3	4 ± 2	5 ± 1
	CON	15 ± 2	4 ± 1	5 ± 1
Day 2	HC	13 ± 2	4 ± 1	5 ± 1
	CON	14 ± 3	4 ± 1	5 ± 1
Day 3	HC	14 ± 2	4 ± 1	4 ± 1
	CON	15 ± 2	4 ± 1	4 ± 1
Day 4	HC	15 ± 4	4 ± 1	5 ± 1
	CON	16 ± 1	4 ± 1	5 ± 1
All Days	HC	14 ± 2	4 ± 1	5 ± 1
	CON	15 ± 2	4 ± 1	5 ± 1

Table 2.16

High School # 2: Perceptual Measures During Activity

	Condition	RPE	Thirst	Thermal
Day 1	HC	12 ± 2	4 ± 1	5 ± 1
	CON	14 ± 2	4 ± 1	5 ± 1
Day 2	HC	14 ± 1	5 ± 1	5 ± 1
	CON	14 ± 2	5 ± 1	5 ± 1
Day 3	HC	14 ± 1	4 ± 1	5 ± 1
	CON	14 ± 1	5 ± 1	5 ± 1
Day 4	HC	14 ± 1	4 ± 1	5 ± 1
	CON	13 ± 1	4 ± 1	5 ± 1
All Days	HC	13 ± 2	4 ± 1	5 ± 1
	CON	14 ± 2	4 ± 1	5 ± 1

Figures

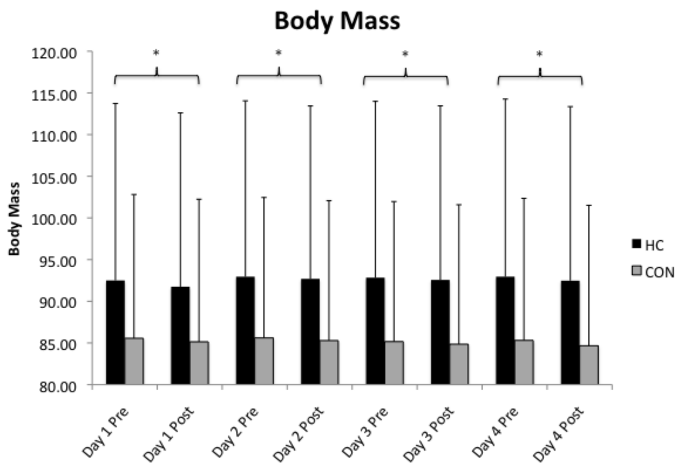

Figure 2.1 Body Mass PRE and POST Practice for High School #1
*** = significant main effect for time compared to PRE**

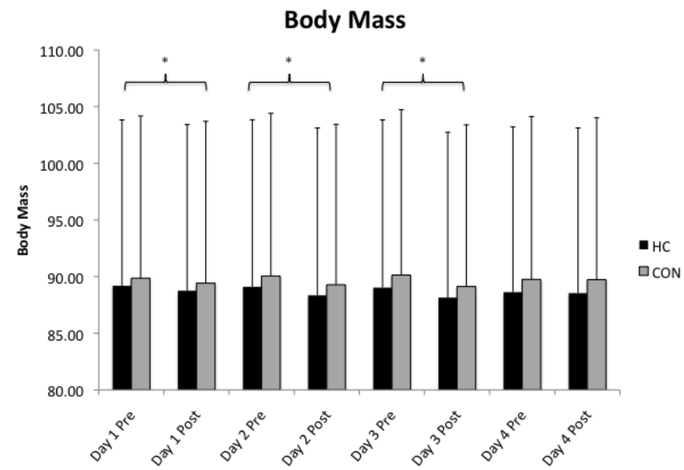

Figure 2.3 Body Mass PRE and POST practice for High School #2
*** = significant main effect for time compared to PRE**

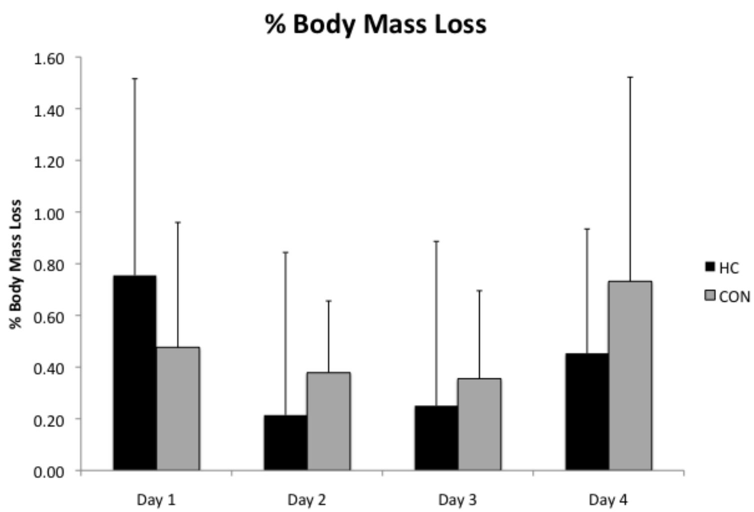

Figure 2.2 %BML over all 4 days of practice for High School #1

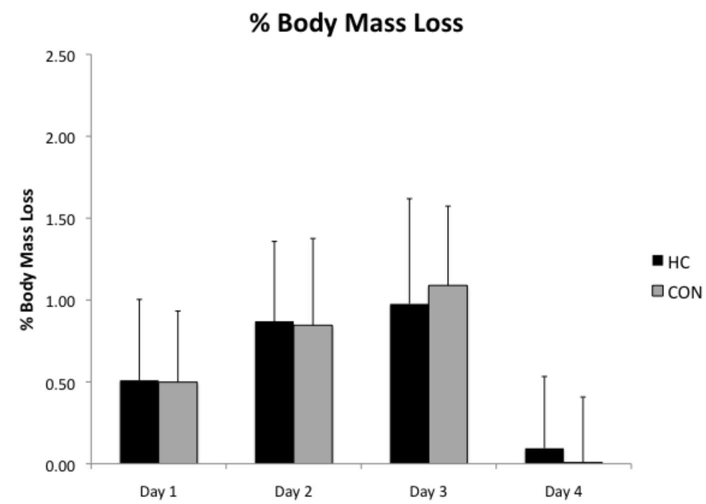

Figure 2.4%BML over all 4 days of practice for High School #2

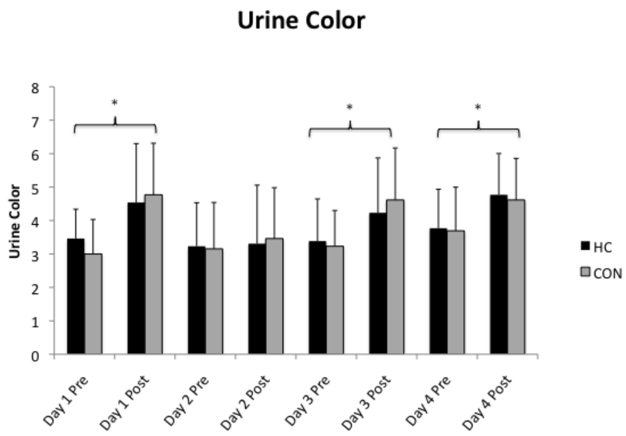

Figure 2.5 Urine color PRE and POST practice
***= significant main effect for time from PRE**

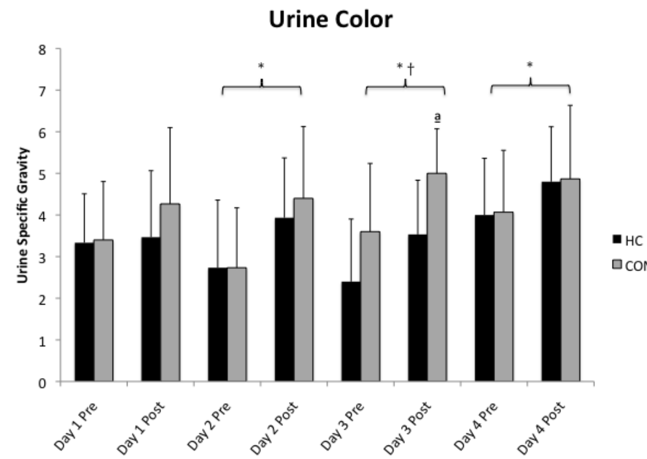

Figure 2.7 Urine color PRE and POST practice
***= significant main effect for time from PRE**

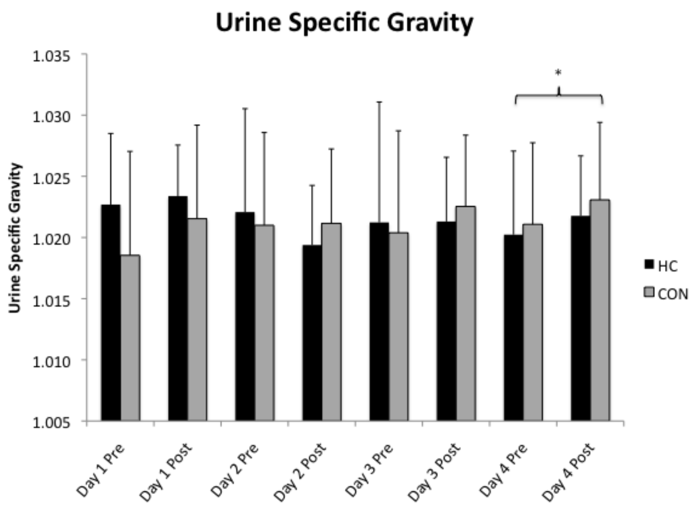

Figure 2.6 Urine specific gravity PRE and POST practice
***= significant main effect for time from PRE**

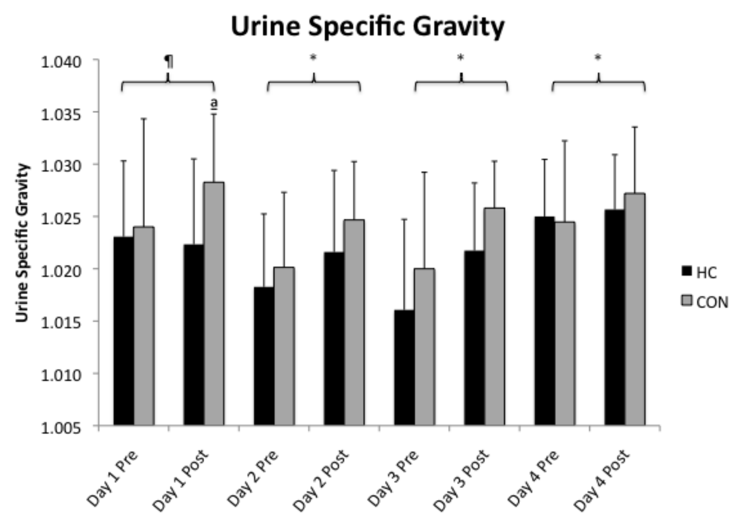

Figure 2.8 Urine specific gravity PRE and POST practice
***= significant main effect for time from PRE**

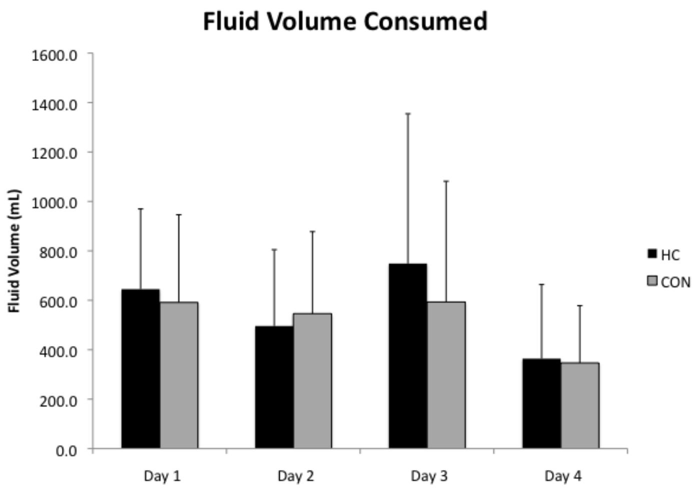

Figure 2.9 Fluid volume consumed during each practice session HS#1

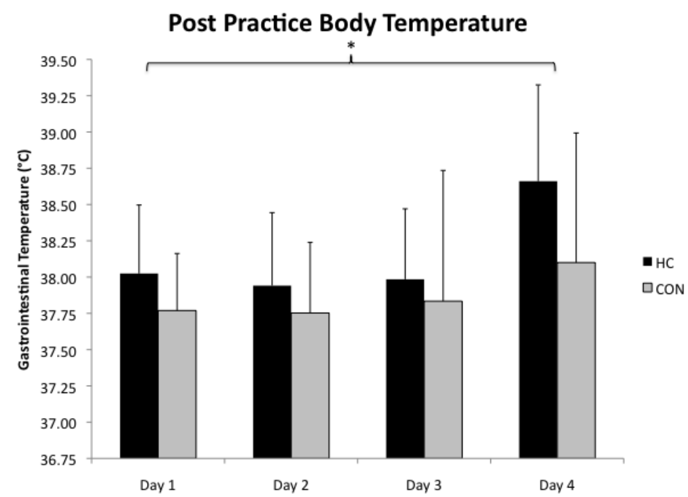

Figure 2.11 POST practice body temperature over all practice days for HS#1
*** = significant main effect for time compared to PRE practice T_{GI}**

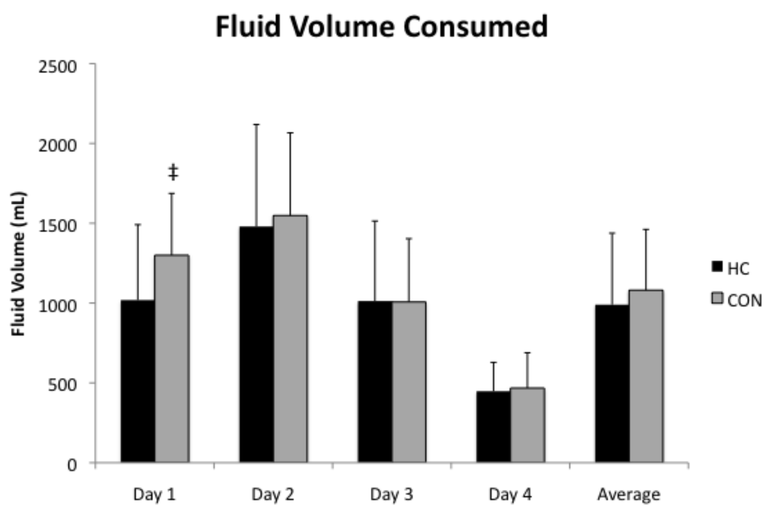

Figure 2.10 Fluid volume consumed during each practice session HS#2

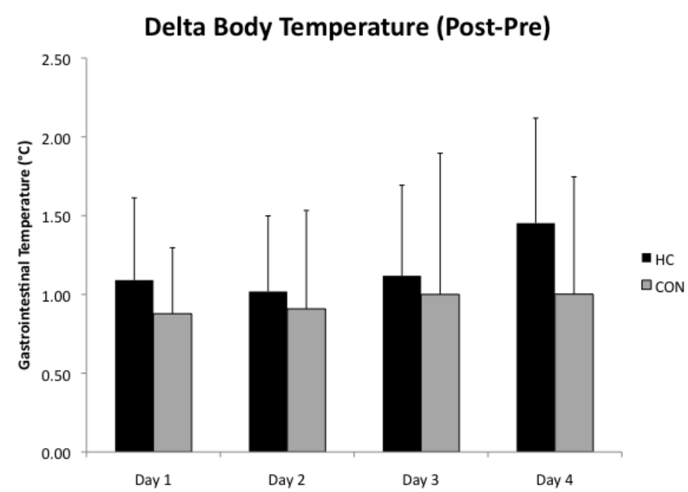

Figure 2.12 Delta T_{GI} over all practice days for HS#1

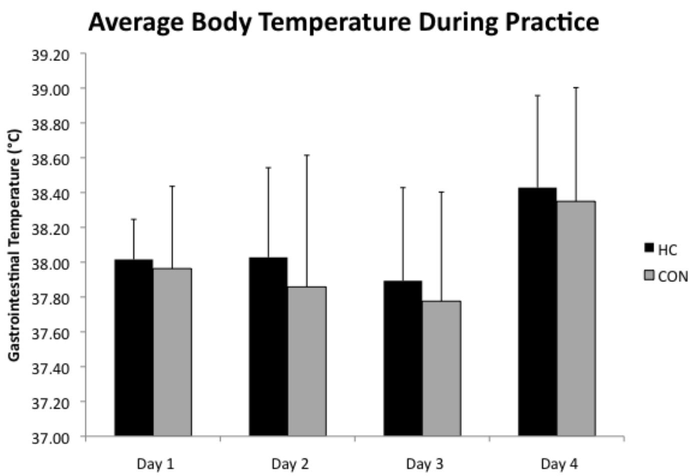

Figure 2.13 Average temperature during practice breaks for HS#1

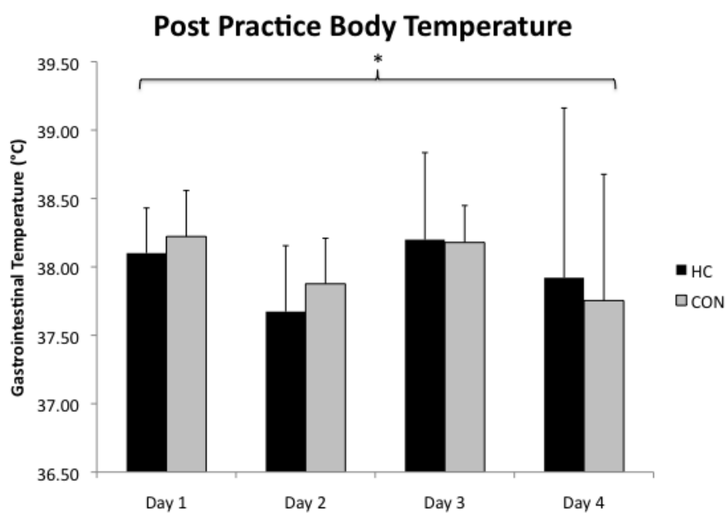

Figure 2.14 POST practice body temperature over all practice days for HS#2
*** = significant main effect for time compared to PRE practice T_{GI}**

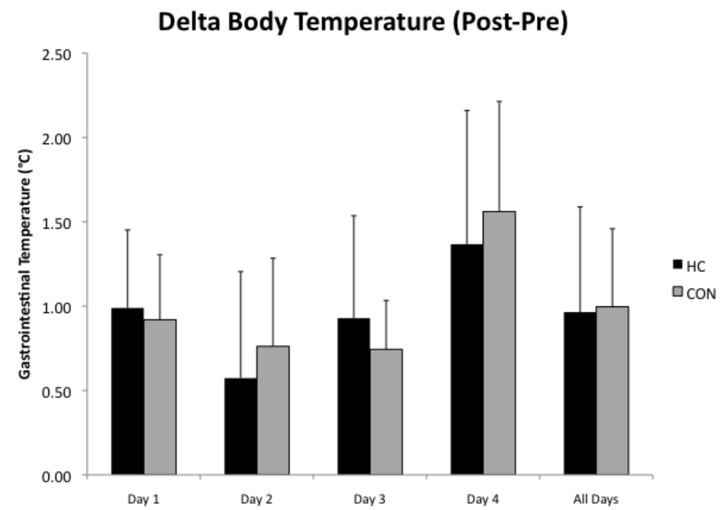

Figure 2.15 Delta T_{GI} over all practice days for HS#2

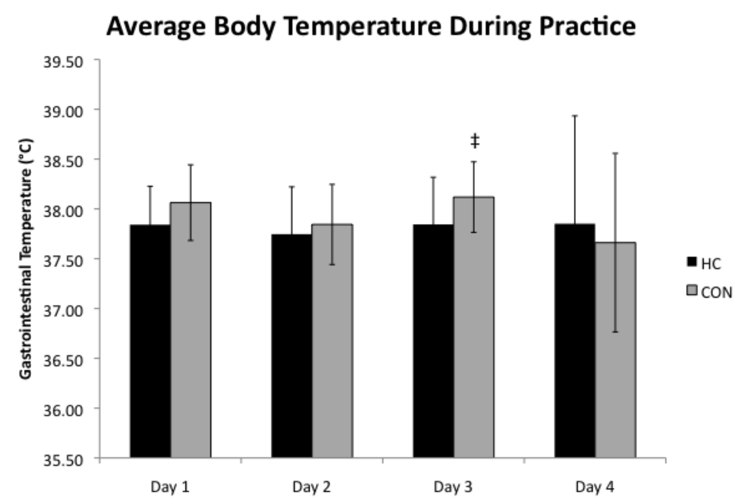

Figure 2.16 Average T_{GI} during practice breaks for HS#2
‡= Moderate effect size compared to HC (=0.65)

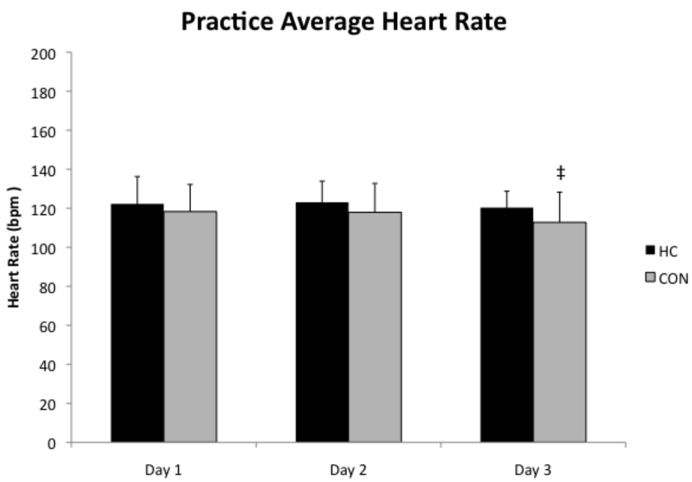

Figure 2.17 Average HR obtained during practice sessions at HS#1
‡ Moderate effect (0.55) compared to HC

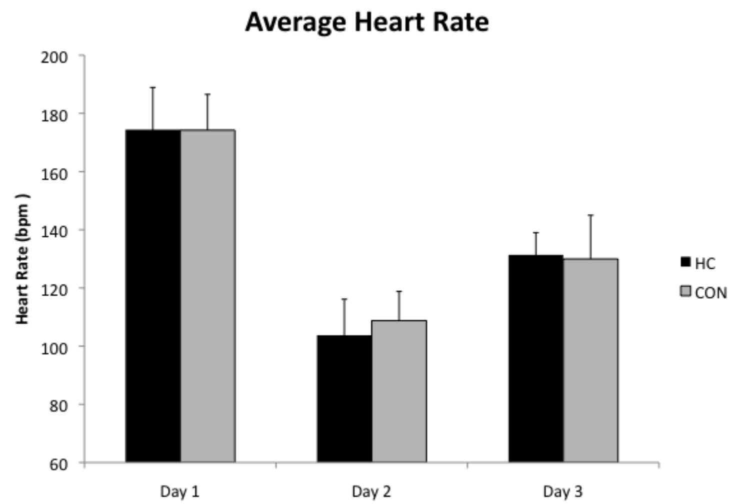

Figure 2.19 Average HR obtained during practice sessions at HS#2

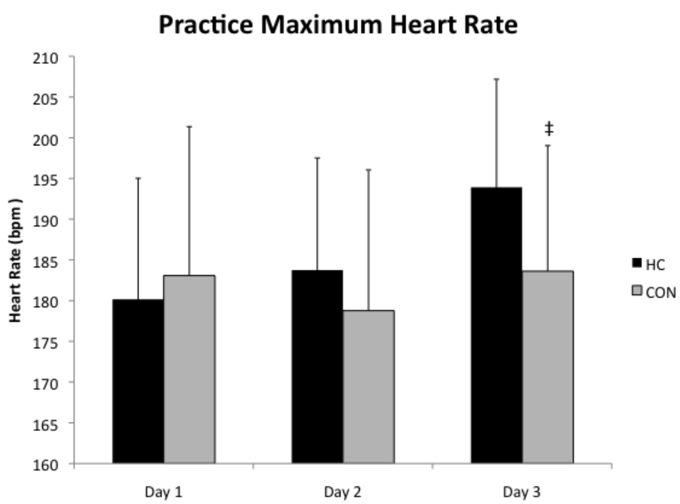

Figure 2.18 HR$_{max}$ obtained during practice sessions at HS#1
‡ Moderate effect (0.71) compared to HC

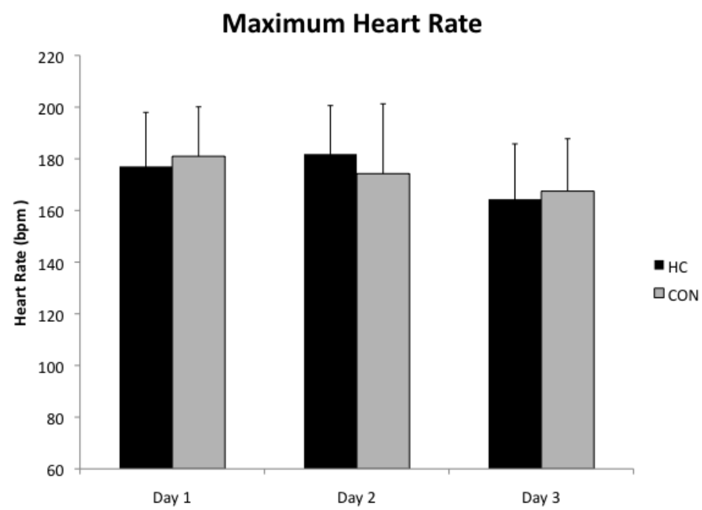

Figure 2.20 HR$_{max}$ obtained during practice sessions at HS#2

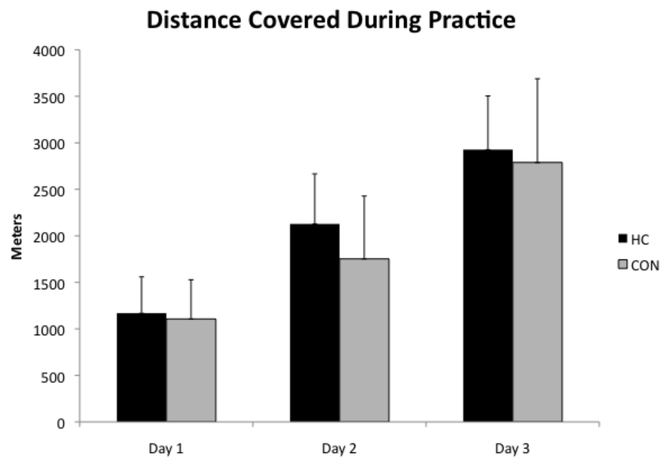

Figure 2.21 Distance covered during each practice session for HS#1

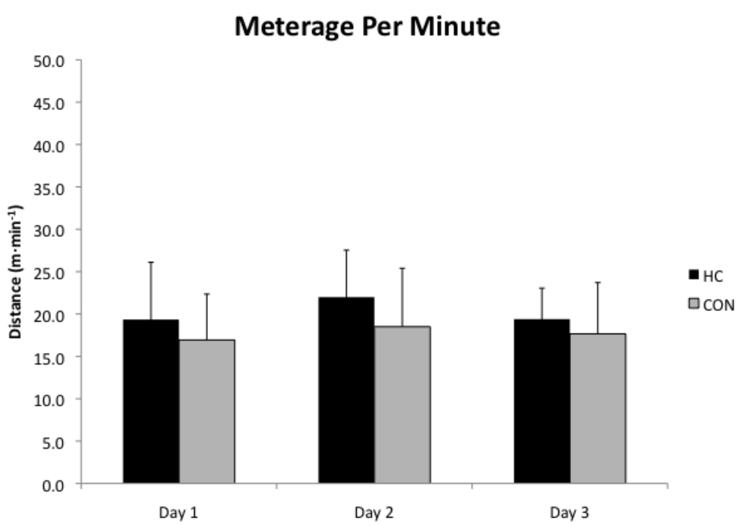

Figure 2.22 Average speed during active practice time for HS#1

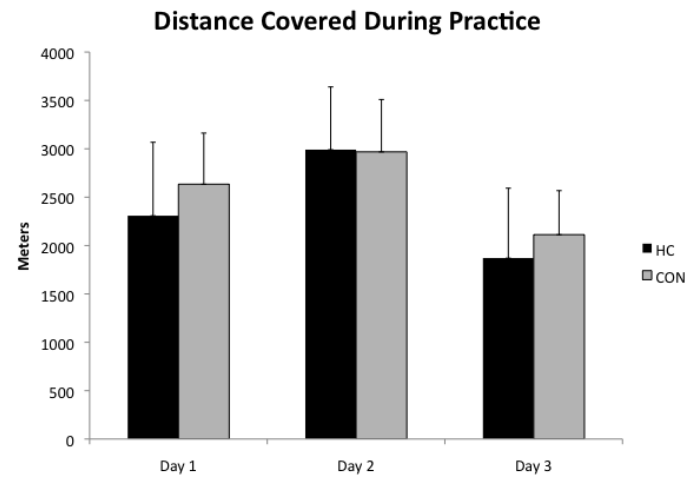

Figure 2.23 Distance covered during each practice session for HS#2

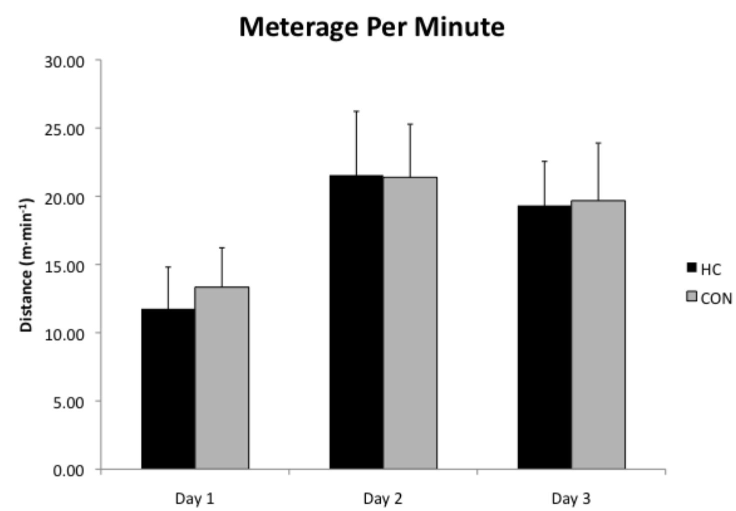

Figure 2.24 Average speed during active practice time for HS#2

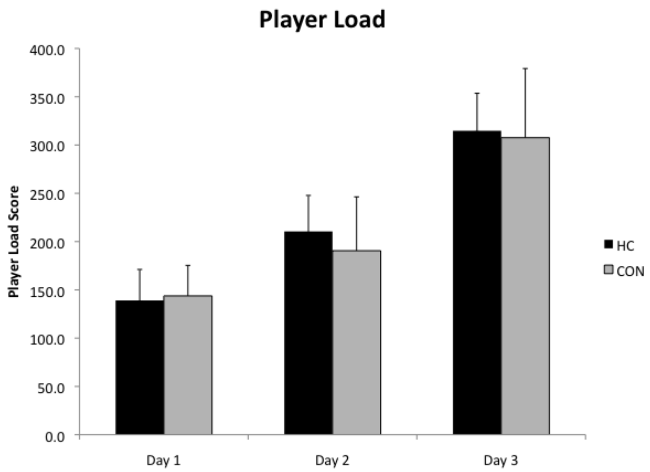

Figure 2.25 Player Load™ accumulated on each day for HS#1

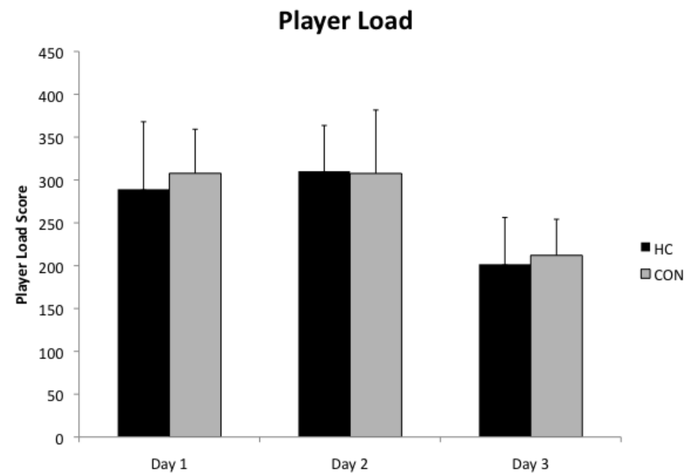

Figure 2.27 Player Load™ accumulated on each day for HS#2

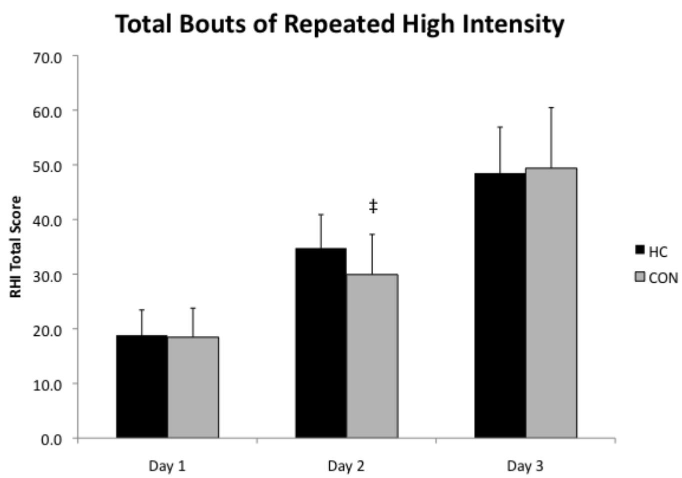

Figure 2.26 Repeated high intensity effort bouts for HS#1
‡ = Moderate effect size (0.77) compared to HC

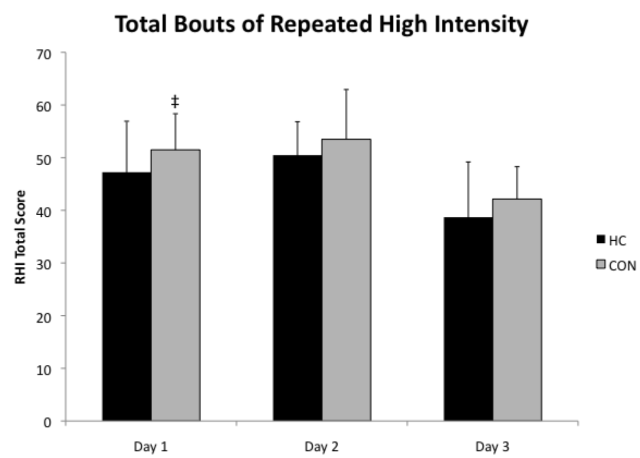

Figure 2.28 Repeated high intensity effort bouts for HS#2
‡= Moderate effect size (0.57) compared to HC

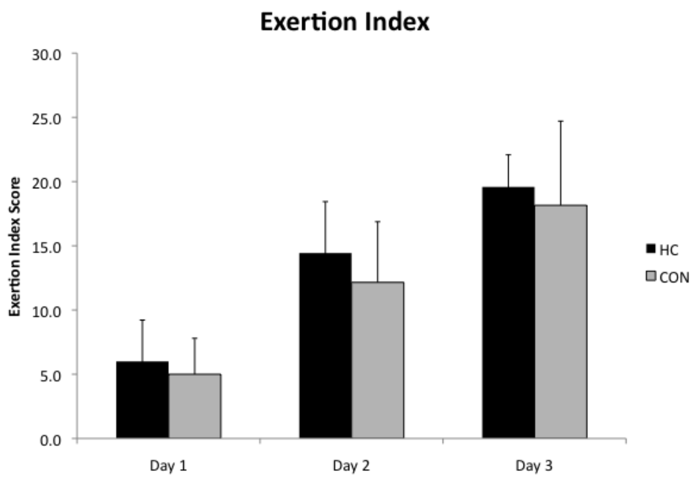

Figure 2.29 Exertion index for all days for HS#1

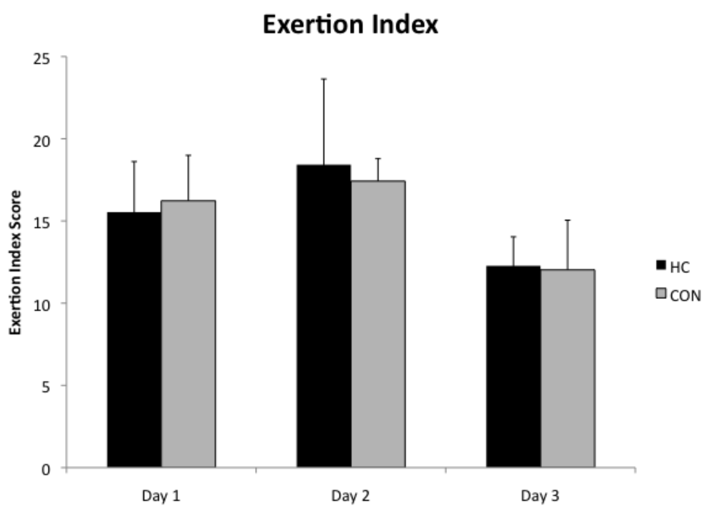

Figure 2.30 Exertion index for all day for HS#2

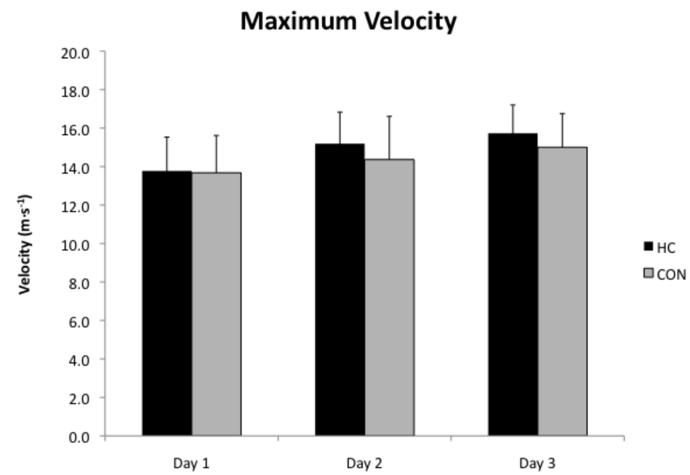

Figure 2.31 Maximum velocity achieved during practice at HS#1

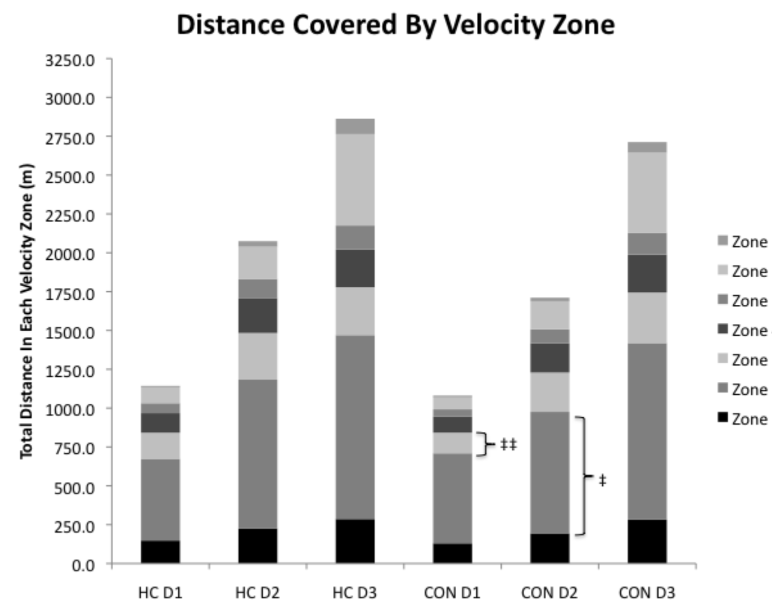

Figure 2.32 Distance covered in meters in each velocity zone for HS#1
‡ Moderate effect size (-0.62) compared to HC D2 for Zone 2
‡‡ Moderate effect size (-0.57) compared to HC D1 for Zone 3

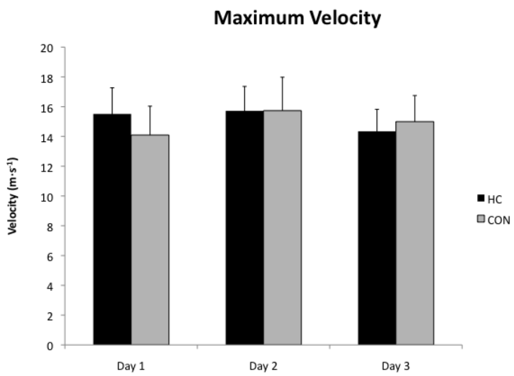

Figure 2.33 Maximum velocity achieved during practice at HS#2

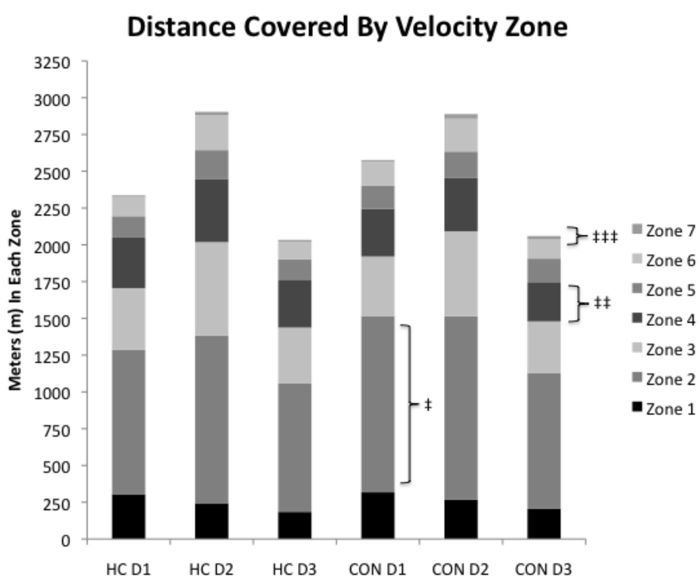

Figure 2.34 Distance covered in meters for each velocity zone for HS#2
‡ Moderate effect size (0.72) compared to HC D1 for Zone 2
‡‡ Moderate effect size (-0.50) compared to HC D3 for Zone 4
‡‡‡ Moderate effect size (0.57) compared to HC D3 for Zone 7

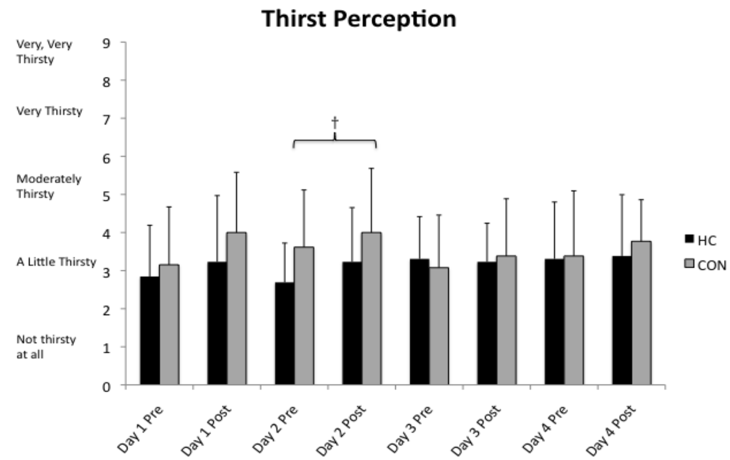

Figure 2.35 Thirst perception at PRE and POST exercise for HS#1
†= significant main effect for group

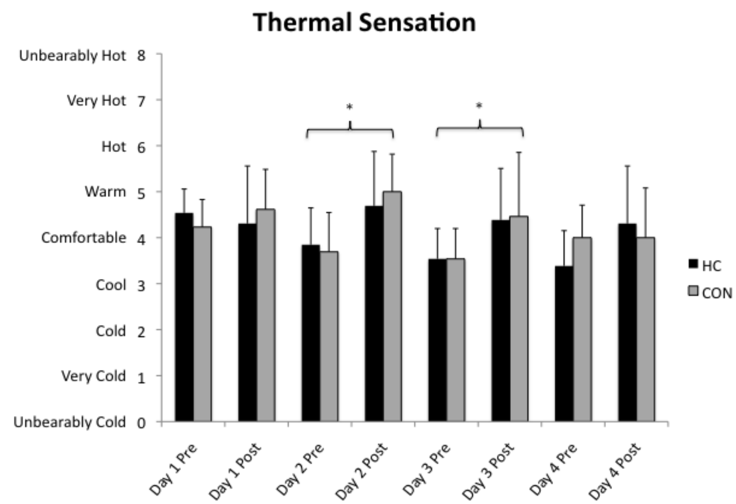

Figure 2.36 Thermal sensation at PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**

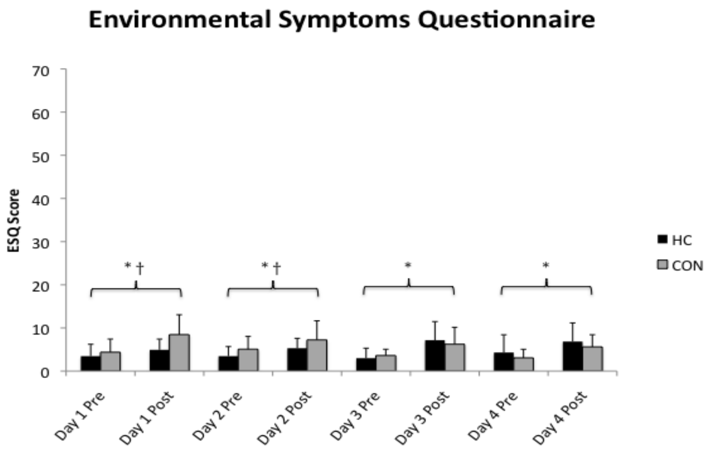

Figure 2.37 ESQ score at PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**
†= significant main effect for group

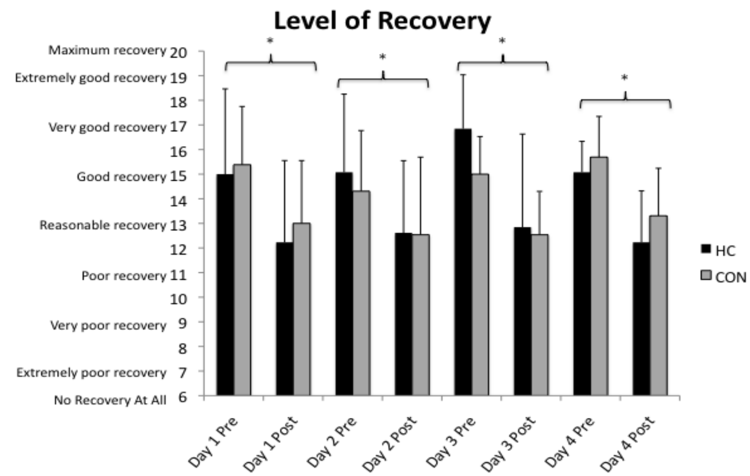

Figure 2.39 Level of recovery perceived PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**

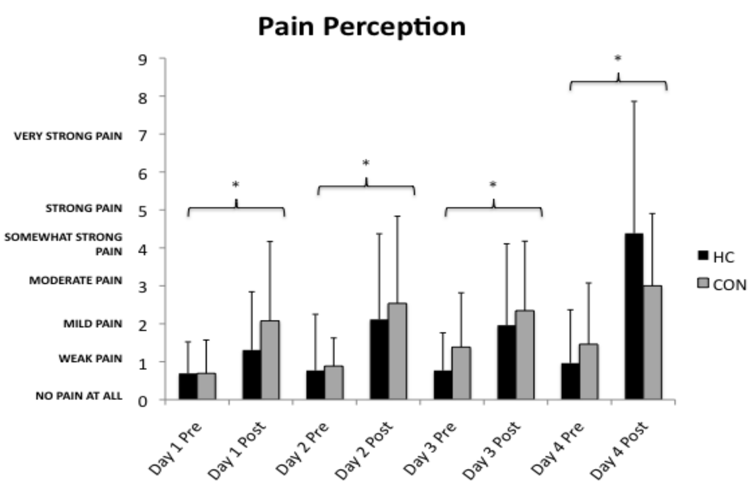

Figure 2.38 Pain perception at PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**

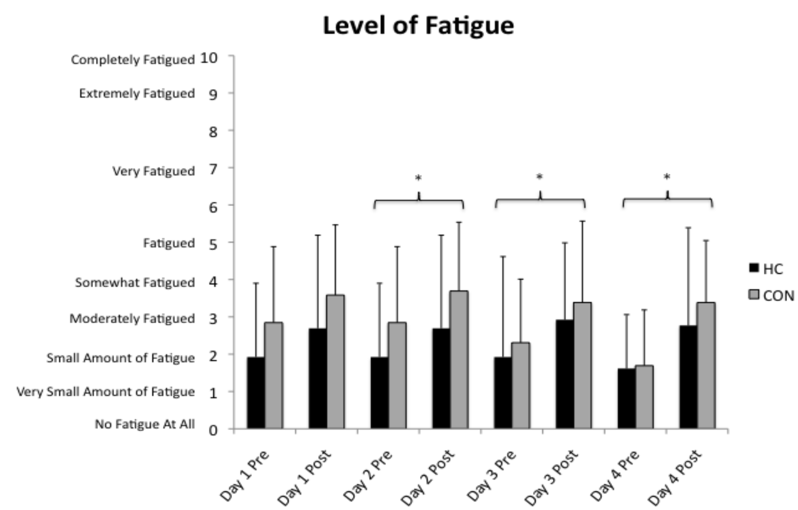

Figure 2.40 Level of fatigue perceived PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**

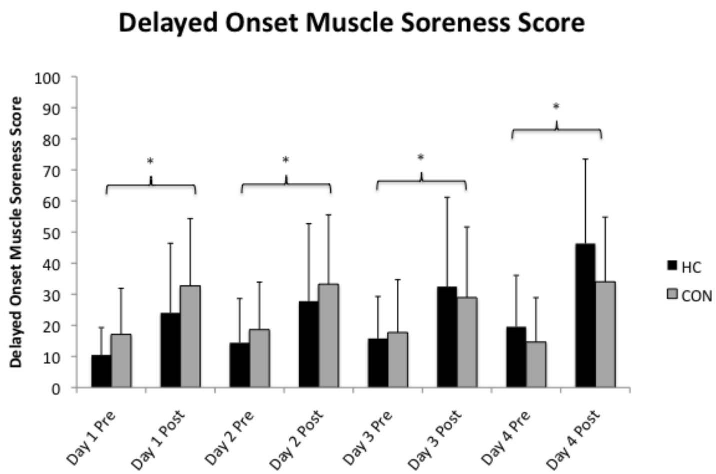

Figure 2.40 Level of perceived DOMS PRE and POST exercise for HS#1
***= significant main effect for time from PRE to POST**

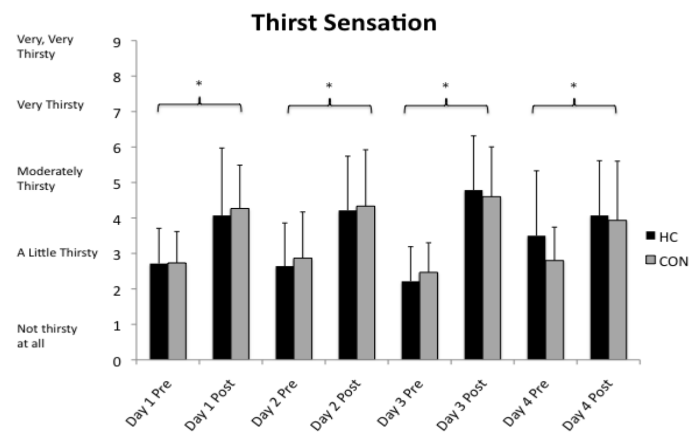

Figure 2.42 Thirst perception at PRE and POST exercise for HS#2
†= significant main effect for group

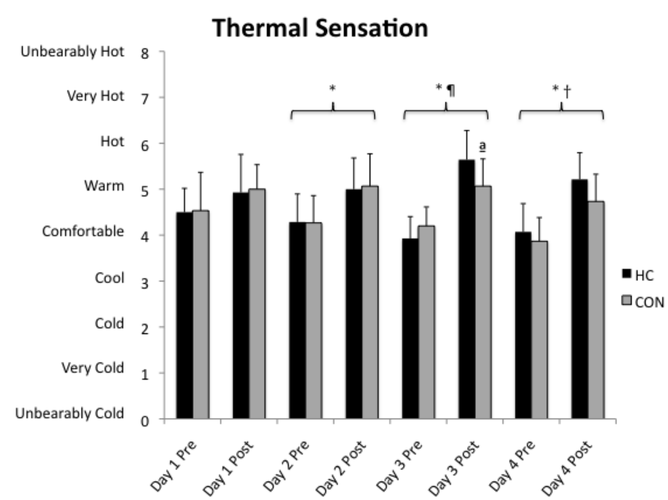

Figure 2.43 Thermal sensation at PRE and POST exercise for HS#2
***= significant main effect for time from PRE to POST**
† Significant main effect for group; ¶ Significant interaction (time x group) ;
ª significantly different than HC

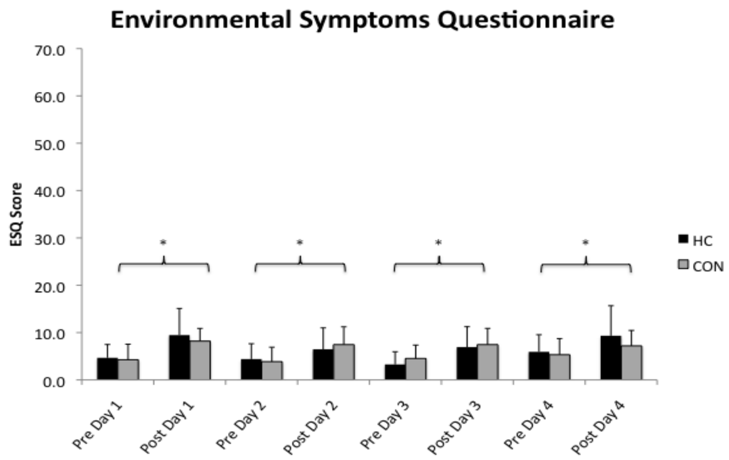

Figure 2.44 ESQ score at PRE and POST exercise for HS#2
***= significant main effect for time from PRE to POST**

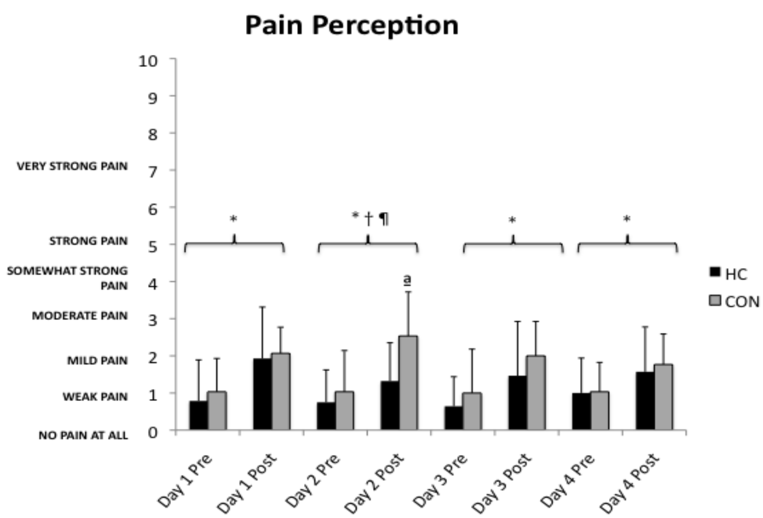

Figure 2.45 Pain perception at PRE and POST exercise for HS#2
*** Significant main effect for time; † Significant main effect for group; ¶ Significant interaction (time x group); ª significantly different than HC**

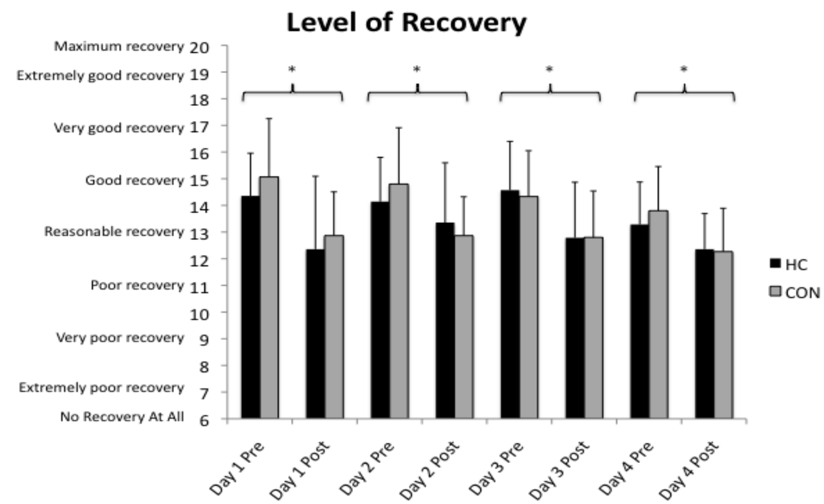

Figure 2.46 Level of recovery perceived PRE and POST exercise for HS#2
***= significant main effect for time from PRE to POST**

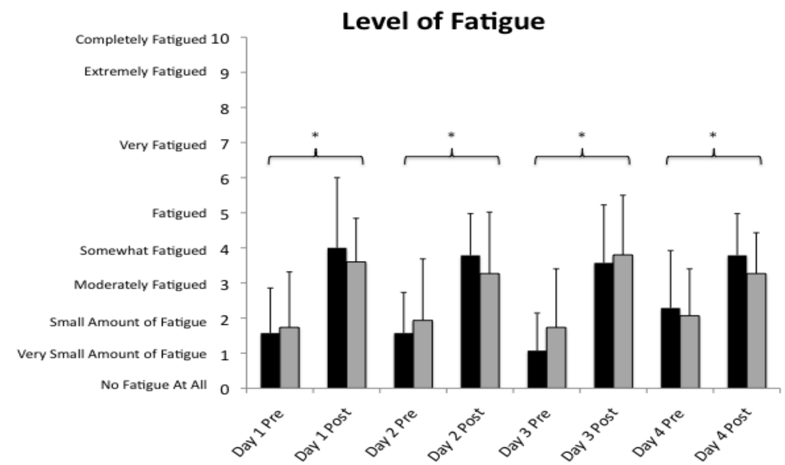

Figure 2.47 Level of fatigue perceived PRE and POST exercise for HS#2
***= significant main effect for time from PRE to POST**

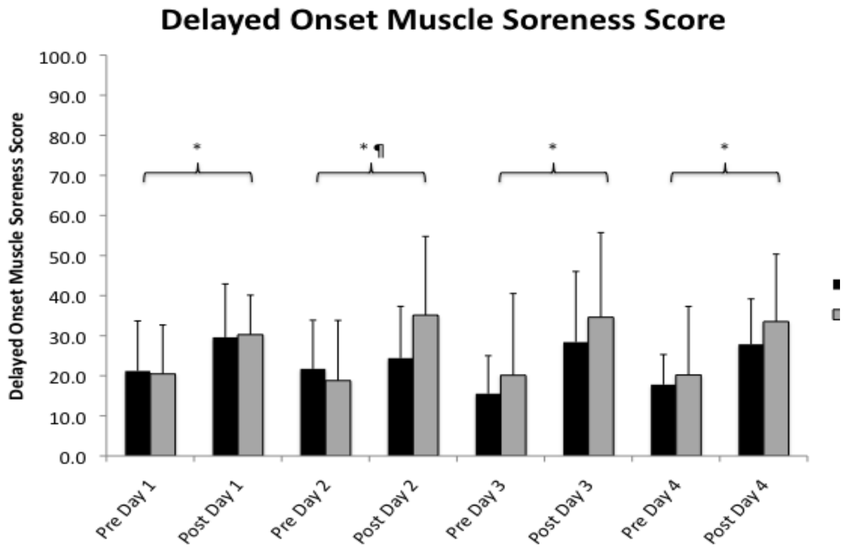

Figure 2.48 Level of perceived DOMS PRE and POST exercise for HS#2
***= significant main effect for time from PRE to POST**

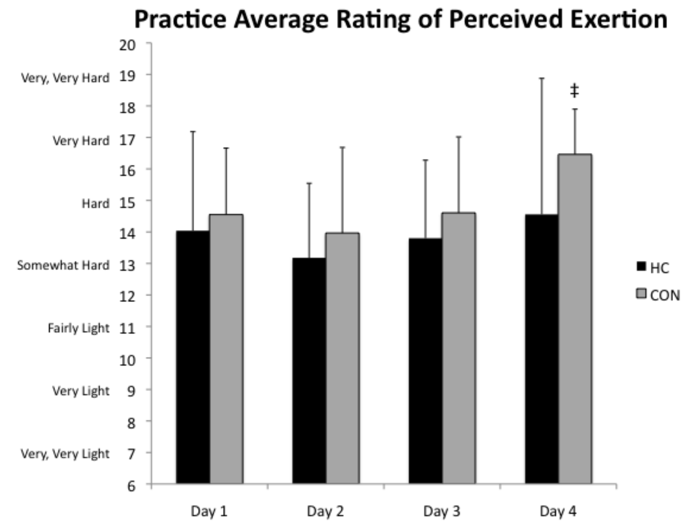

Figure 2.49 Rating of perceived exertion during exercise breaks for HS#1
‡ = Moderate effect size (0.69) compared to HC

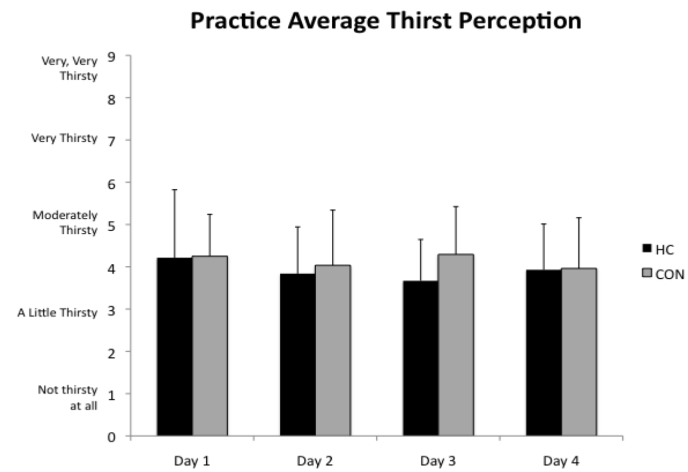

Figure 2.50 Thirst perception during exercise breaks for HS#1

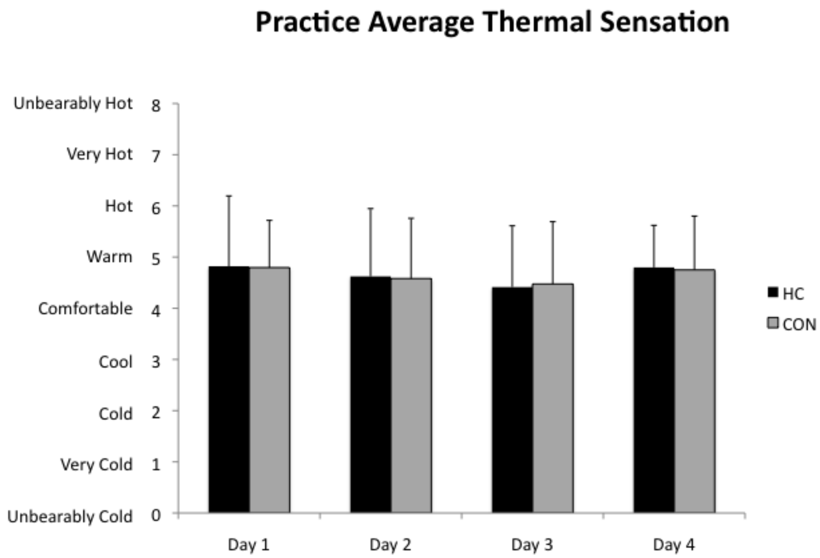

Figure 2.51 Thermal sensation perceived during exercise breaks for HS#1

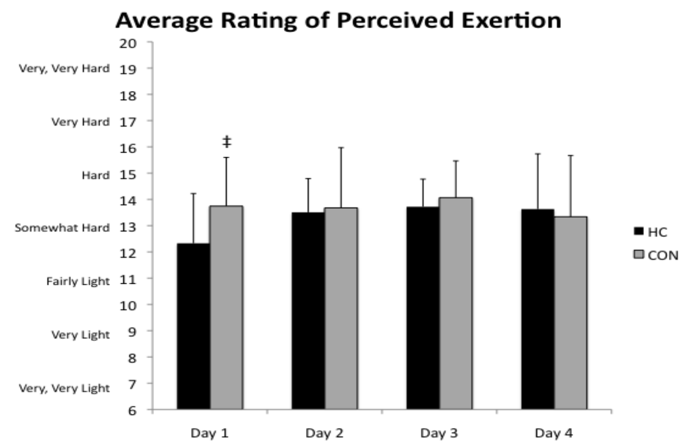

Figure 2.52 Rating of perceived exertion during exercise breaks for HS#2
‡ = Moderate effect size (0.74) compared to HC

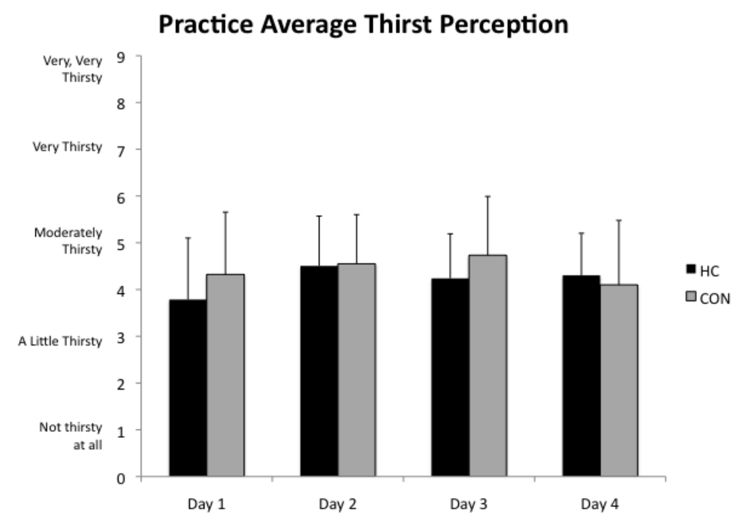

Figure 2.53 Thirst perception during exercise breaks for HS#2

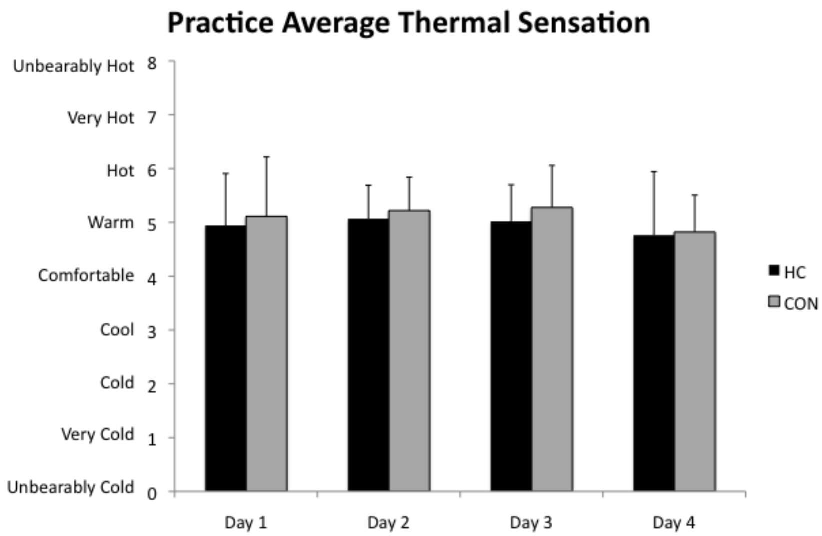

Figure 2.54 Thermal sensation perceived during exercise breaks for HS#2

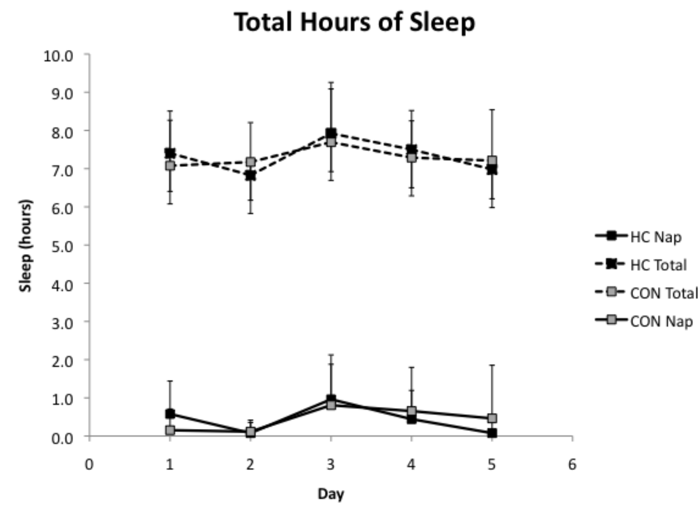

Figure 2.55 Total hours of sleep and nap hours each day for HS#1

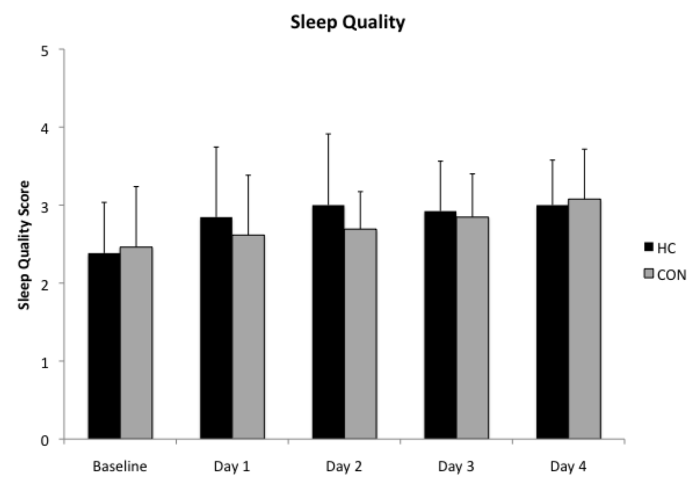

Figure 2.56 Level of perceived sleep quality each day for HS#1

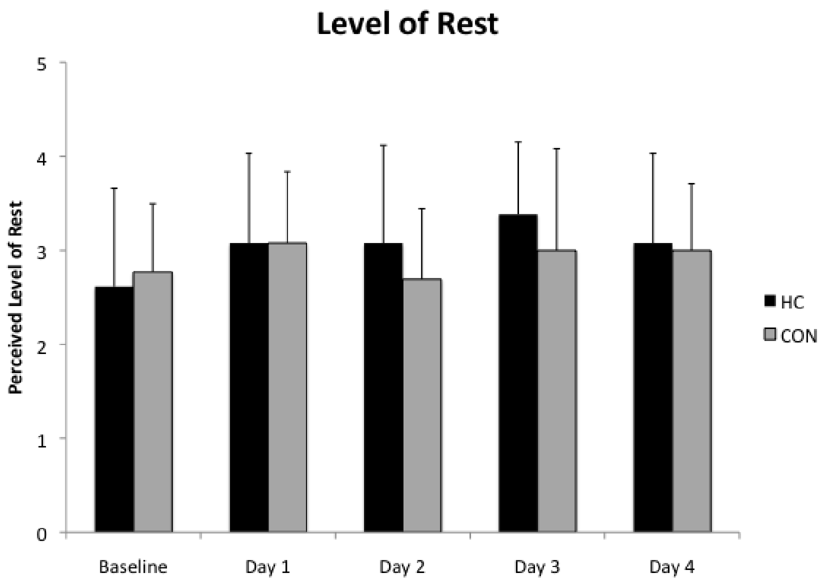

Figure 2.57 Level of perceived rest by day for HS#1

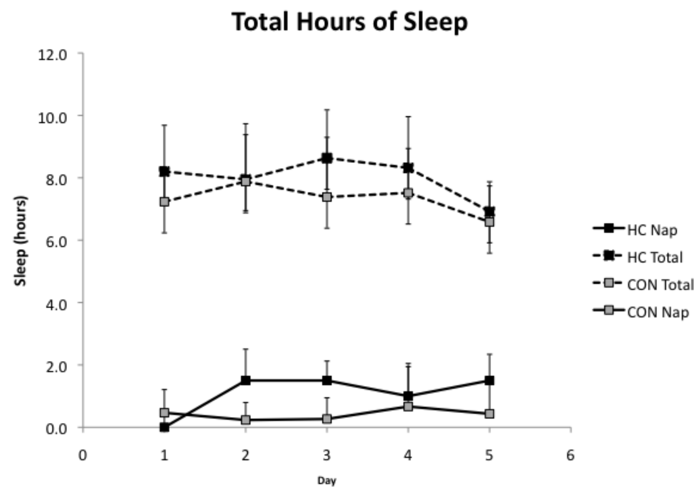

Figure 2.58 Total hours of sleep and nap hours each day for HS#2

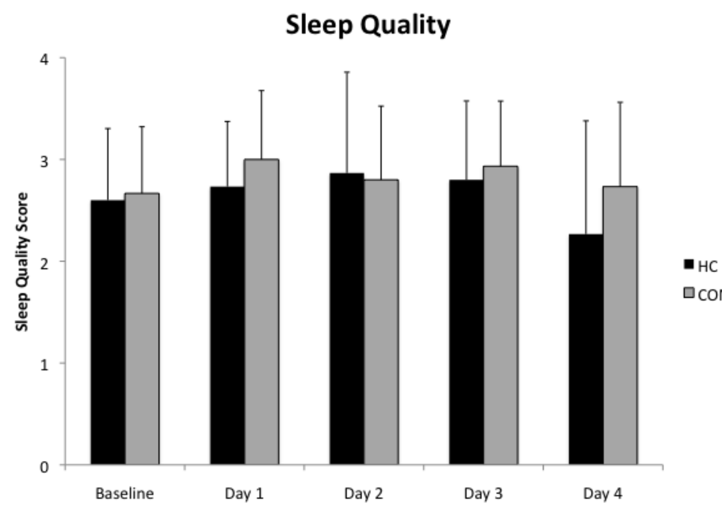

Figure 2.59 Level of perceived sleep quality each day for HS#2

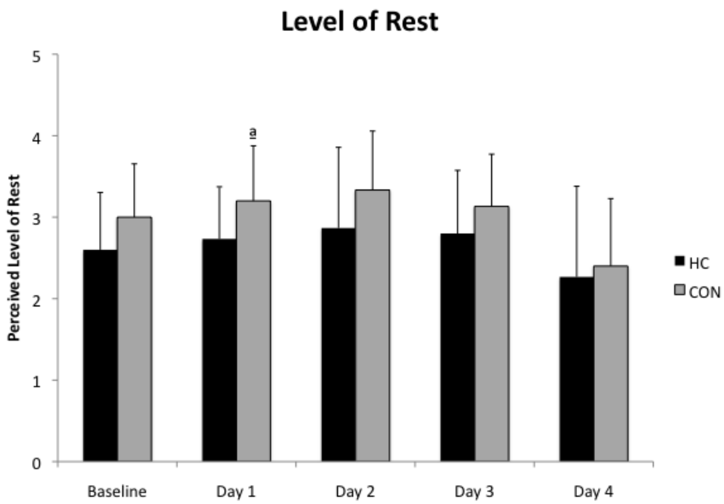

Figure 2.60 Level of perceived rest by day for HS#2
[a] significantly different than HC (p= 0.005)

CHAPTER 3: THE INFLUENCE OF HAND COOLING AND HYDRATION ON BODY TEMPERATURE DURING UNCOMPENSABLE HEAT STRESS AND THE INFLAMMATORY CYTOKINE RESPONSE

ABSTRACT

Various methods of cooling effectively reduce body temperature in hyperthermic individuals. The effect of cooling on circulating inflammatory cytokines and stress hormones is well established but specific effects of peripheral cooling of the hand are not well elucidated. **Purpose:** Our aim was to measure how circulating inflammatory cytokines (interleukin (IL)-6, IL-12, IL-1B (receptor agonist), IL-8, IL-10, tumor necrosis factor (TNF)- , IL-12p70 and hormones testosterone (T), cortisol (C), and creatine kinase (CK) respond to peripheral hand cooling with fluid (HCF), hand cooling (HC) and control (CTRL). **Participants:** Twelve males ((mean ± SD) age: 24 ± 3yrs, height: 179 ± 5cm, body mass: 82.64 ± 9.77kg) Intervention: Participants completed three 90-min treadmill exercise trials while wearing a full American football uniform in a hot environment (39°C, 40%rh). Every 12th minute of exercise, one hand was cooled for 3 minutes. **Main Outcome Measures:** We measured rectal temperature (Tre) and heart rate (HR) before (PRE), during (every 12th min), and after (POST) exercise. We collected blood samples, urine specific gravity, and body mass PRE and POST exercise. We measured plasma cytokine concentrations using a multiplexed cytometric bead array (CBA) and plasma hormones with standard ELISAs. Statistically significant differences ($p < 0.05$) were determined using separate two-way repeated measures ANOVA for (condition x time) with post-hoc Bonferroni correction. **Results:** Subjects were more dehydrated in HC and CTRL compared to HCF (HCF= 1.60±0.53%, HC=3.08±0.59%, CTRL=3.29±0.68%, p= 0.001). Rectal temperature (Tre) significantly changed over time and was different

between groups at specific time points (main effect for time and condition x time interaction $F_{38,418}$= 2.674, p=0.049, ^{2}= 0.196). In particular, Tre peaked at 39.24±0.49℃ in CTRL(39.24±0.49℃) and was higher than HC (38.86±0.45℃) and HCF (38.64±0.39℃). Elevations in HR also existed ($F_{2,22}$= 4.55, p=0.044, ^{2}= 0.293) indicating that the stress of exercise increased substantially. Elevations in testosterone ($F_{1,10}$= 38.53, p= 0.001, ^{2}= 0.794)and CK ($F_{1,10}$= 38.82, p= 0.001, ^{2}= 0.784) suggest that the muscle damage and endocrine responses were elevated. Substantial elevations in C (3-15%), T (33-36%), and CK (34-48%) indicate the degree of stress during the protocol. Inflammatory markers IL-8 and IL-6 were different over time (IL-8: $F_{1,10}$= 83.74, p= 0.001, ^{2}= 0.893; IL-6: F1,10= 20.73, p= 0.001, ^{2}= 0.675). Group differences were present for IL-8 (IL-8: $F_{2,20}$= 10.50, p= 0.001, ^{2}= 0.512) and •IL-8 responses were increased for the HC group compared to the HCF group (p=0.008). **Conclusion:** During uncompensable exercise while wearing football equipment hand cooling with fluid resulted in lower body temperature, less dehydration (reduced %BML) and moderate reductions in stress and inflammation. Hand cooling and fluid replacement successfully resulted in moderate to large reductions in circulating cortisol, IL-6, and IL-8 suggesting that this treatment approach reduces physiological stress and mitigates the inflammatory response while wearing football attire.

INTRODUCTION:

Cytokines are intracellular signaling peptides critical to proper inflammatory response, repair, and adaptation. They are released into the circulation in response to a stress. Since 1983[1] commonly studied cytokines in exercise and environmental stress include interleukin (IL)-6, IL-12, IL-1β, IL-1ra (receptor agonist), IL-8, IL-10, IL-2, and tumor necrosis factor alpha (TNFα) among others.[2–4] Cytokines are either pro-inflammatory or anti-inflammatory in nature and modulate the inflammatory response to tissue damage. IL-6, IL-10, IL1ra, and TNFrI & II are anti-inflammatory, while IL-1a, IL-1β, IL-2, and TNFα among others are pro-inflammatory. The balance between the pro- and anti-inflammatory responses is critical to stress response, adaptation, and resilience. Inflammation is important and critical to understand during exercise and heat stress.

The inflammatory response to exercise and hyperthermic stress is well understood.[2,3,5] Many researchers have studied circulating cytokine responses to controlled exercise in the heat[5–9] military training[10] and marathons.[7,8] The acute and chronic stress associated with these activities initiates the inflammatory response in an attempt to re-establish homeostasis.[7,11] These mediators known as cytokines are released in an attempt to maintain the homeostatic environment of the cell and prepare for future stress induced responses. When there is a loss in control or an unwarranted response occurs (e.g. extreme stress) this can result in a systemic inflammatory response as described by Bouchama and Knochel.[5] This hypercytokinemia can lead to acidosis, impaired liver function, and ensuing endoxemia. In situations related to excessive exercise associated hyperthermia (e.g. exertional heat stroke) this endotoxic response may lead to encephalopathy, renal failure, rhabdomyolysis, cerebral ischemia, myocardial infarction, and subsequent mortality if left untreated. Currently, these cases have been observed during pilgrimages to Mecca, military training and combat, the workplace, and the sport of American football. Reduction in heat stress through cooling modalities may help regulate inflammation.

The modulation of inflammatory cytokine release has been successful through the "thermal clamping" technique. Thermal clamping by using water bath immersion during exercise limits exercise-associated temperature increases to <0.5°C and significantly reduces stress hormones and inflammatory cytokines[12] This suggests that cooling modalities mitigate the rise in body temperature during exercise and heat stress and so regulate the ensuing inflammatory response to exercise and environmental stress.

The present study was undertaken to examine the circulating inflammatory and hormonal response to hyperthermic exercise in the heat while wearing American football attire and to examine the potential influence of cooling with fluid replacement, and cooling alone on these responses. We hypothesize that reductions in body temperature will occur through the use of hand cooling with fluid replacement and that hormonal stress and cytokine inflammatory responses will be decreased due to reduced physiological stress compared to cooling alone and to the control condition.

METHODS:

Participants

We recruited twelve college-aged male participants from the University of Connecticut (age = 24±3 years, height= 179±5 cm, body mass = 82.64±9.77kg, body fat = 18.61±6.71%, lean mass = 77.88±6.37%) in the analysis. Female participants were not recruited in order to avoid fluctuations in cytokine and hormonal responses due to menstrual cycles[13,14] and contraceptive use.[15]

All participants completed a medical history questionnaire and were excluded if any of the following criteria were met: (1) previous history of exertional heat stroke in the last 3 years, (2) intolerance to the heat, (3) cardiovascular, metabolic or respiratory disease, (4) medication or dietary supplements known to alter thermoregulation, or (5) use of tobacco products. All participants provided written informed consent and the research was approved by the Institutional Review Board for Human Studies. One subject's values were removed from the analysis for the plasma [testosterone] and the inflammatory cytokine panel because their values were outliers. Therefore the number of subjects included in the analysis for these variables was n=11.

Design

All participants performed a familiarization day including body-composition testing via DEXA scanner, sweat rate measurement to determine fluid replacement needs for the HCF trial, instruction in the exercise protocol, familiarization to the hand cooling treatment, and fitting of the football equipment (helmet, shoulder pads, pants, and jersey). After this initial visit they returned 5-7 days later for their first trial. All trials were standardized to the same time of day and separated by 5-7 days for all participants. The order of trial conditions were counterbalanced and randomized for the HCF, HC, and CON conditions.

Following a 10 minute equilibration period in the heat (39°C, 40%rh) blood samples were collected at the pre-exercise time point (PRE) and immediately post-exercise (POST). All samples were collected in the standing position and within 5 minutes of both the commencement/completion of exercise.

All participants drank 500 mL of water the evening before and the morning of testing to ensure euhydration. Additionally, participants were asked to replicate their exercise regimen and diet for the 24 hours leading up to the trials and to also complete a diet and fluid record.

Testing Protocols:

Familiarization Session

Participants were asked to arrive in the same clothing and shoes that they would wear for each session. First, body fat mass and lean mass were assessed using a DEXA scanner (GE Lunar Prodigy Dual X-Ray Absorptiometry, General Electric Fairfield, CT). Participants were then asked to provide a urine sample and to void their bladder and insert a calibrated rectal thermometer (YSI Spring Instruments, TX) 10 cm past the anal sphincter. Body mass was obtained using a calibrated scale (model BWB-800A; Tanita Corp, Tokyo, Japan) and hydration status was assessed using a hand held refractometer (Atago 300 CL, Atago, Japan). All participants began exercise if urine specific gravity (USG) $\leq$ 1.020. Participants were then fitted with a heart rate monitor (Timex digital 2.4 heart rate monitor, Timex Group USA, Middlebury, CT) and standard American football attire. Each participant wore a Football Helmet (Riddell; Speed), shoulder pads (Riddell; Power CPX 30), football pants (Nike; Team Apparel [with internal tailbone, Hip (Adams USA), Thigh (Bike) and Knee (Schutt) pads]) and football jersey (Nike; Team Apparel) over top of the shoulder pads. The sweat rate measurement to determine fluid replacement needs was performed using an abbreviated 30-minute exercise protocol via pre and post body mass loss. The exercise consisted of walking on a motorized treadmill (Precor, Woodinville, VA) in an environmental chamber (model 2000; Minus-Eleven, Inc, Malden, MA) at 40℃; 39% rh. As a part of a larger study, participants were familiarized to a 15-20 minute performance battery consisting of a counter movement vertical jump, 30 second agility and reaction testing, a 20 second sprint on a non-motorized treadmill and balance test. During testing sessions, this battery was performed before and after the treadmill exercise; and is included in the "post exercise" blood sample was taken after this testing battery.

Exercise Protocol

Upon arrival participants again provide a urine sample in to a clean, inert, plastic container and insert the rectal thermistor. Urine was analyzed for color and specific gravity to confirm euhydration (• 1.020) and resting T_{re} was obtained to ensure normal resting body temperature and that the thermometer was working. Body mass while wearing underwear only was obtained on an electronic scale. Participants then were fitted for a HR monitor and the football equipment was applied. Participants entered the chamber and stood quietly for 10 minutes to allow for stabilization to the environment which was maintained throughout all trials at 40.0℃ ± 1.0℃ and a relative humidity of 38.0% ± 1.0% followed by a standing blood sample collection. Participants performed the performance battery associated with a larger study and then completed 90 minutes of exercise on a motorized treadmill at each participants comfortable walking pace (range 1.56-2.01 $m \cdot s^{-1}$ [3.5-4.5mph]; which was determined during the familiarization visit at a 5% grade. All enrolled participants completed the 90 minutes of exercise. During the exercise, heart rate and rectal temperature were obtained every 6 minutes and following cooling treatments via digital heart rate monitor and rectal thermistor respectively.

Hand Cooling Protocol

During the 90 minute treadmill exercise, a hand cooling device (Core Control, Avacore Technologies; Ann Arbor, MI) designed to circulate cold water maintained at (16.4℃ [60℉]) into bladders surrounding the hand with negative sub atmospheric pressure (-40mmHg) and air-tight seal was applied. The hand cooling was applied during the last 3 min of each 15 min exercise block. Participants were asked to exit the treadmill at min 12 and place their right hand into the device (used according to manufacturer specifications) while in a seated position with their helmet removed. The timing of the breaks was according to typical football work to rest ratios during play. During the CON condition, players were asked to place their hand in the device however the device was not turned on. The researcher responsible for all data analysis exited the chamber during each treatment bout and was thus blind to the treatment until the code was provided after completion of data analysis. In addition to hand cooling, one trial included fluid replacement based on the sweat rate calculated during the familiarization visit. The total projected fluid losses (256mL ± 6mL; range 110mL to 346mL) via sweat was divided into 6 equal boluses of room temperature water (21°C [70°F]) and was administered during each 15 min bout of exercise. Participants were instructed to finish the water prior to the 3 min treatment. Upon completion of the 90 min exercise bout, participants performed the performance battery another blood sample was obtained.

Blood Collection Procedures

All blood samples were obtained using an aseptic butterfly needle (21 gauge) equipped with an extension tube and hub (BD Vacutainer Safety-Lok Blood Collection Set, Becton Dickinson, Franklin Lakes, NJ). This was connected to a chilled 5-mL EDTA blood collection tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Blood was allowed to clot and immediately centrifuged at 3000rpm for 20 minutes at 4°C and stored at -80°C until analysis.

Analysis of Samples

The intra- and inter-assay coefficients of variation (CVs) the inter-assay CVs were < 5.0% for all analyses. Plasma was analyzed for testosterone concentration in duplicate by enzyme linked immunoassay (Testosterone ELISA Calbiotech, Spring Valley, CA). Plasma cortisol concentration was analyzed in duplicate using ezyme linked immunoassay (Cortisol ELISA Calbiotech, Spring Valley, CA). Plasma CK concentrations were analyzed in duplicate using

(Creatine Kinase-SL Assay, Sekisui Diagnostics, Charlottetown, PE, Canada). Absorbance was measured on a plate reader (VersaMax; Molecular Devices, Sunnyville, CA).

Inflammatory cytokines were analyzed by flow cytometric (BD Accuri C6, BD Biosciences, Mississauga, Canada) bead array according to manufacturer instructions. (CBA Human Inflammatory kit, BD Biosciences, Mississauga, Canada) in order to detect IL-6, IL-12p70, TNFα, Il-10, IL-1β, and IL-8. Analysis was completed using BD FACSArray Software.

Statistical Analysis

Comparisons of means for were performed using separate two-way repeated measures (condition x time) analysis of variance for physiological variables and for serum concentrations using SPSS (Version 21.0; SPSS Inc, Chicago, IL). Significance was set at p•0.05. A *t*-test post hoc comparison with Bonferroni correction was conducted to determine differences within or between conditions when condition x time interactions were present. Delta (•) values were calculated (POST-PRE) for many of the variables. Data presented were also compared using mean differences (MD), 95% confidence intervals (95%CI), and effect sizes (ES) using cohen's *d.* Data are presented as mean±SD, unless otherwise noted.

RESULTS

Baseline Measures

All participants arrived for testing in a euhydrated state as indicated by urine specific gravity on the familiarization and prior to the start of each testing day (1.016 ± 0.007). Resting rectal temperature on the familiarization day (37.17 ± 0.28°C) and prior to the start of each testing day (37.19 ± 0.29°C) indicated that all participants were normothermic prior to the start of exercise.

Body Mass

Participants' PRE body mass for HCF, HC and CTRL were 82.33 ± 10.00kg, 82.28 ± 9.91kg, and 82.05 ± 10.12kg while POST body mass was 81.02 ± 9.91kg, 79.77 ± 9.86kg, and 79.34 ± 9.68kg for HCF, HC, and CTRL respectively. Independent of time, we observed differences between conditions for body mass ($F_{2,22}$=125.01, p=0.001, η^2 =0.919, observed power= 1.000). Independent of condition, we observed differences between time points for body mass ($F_{1,11}$=32.84, p=.0001, η^2 =0.749, observed power= 0.999). No significant time x group interaction occurred ($F_{2,22}$ = 2.335, p= 0.120, η^2 =0.175, observed power= 0.422). Percentage of body mass loss (%BML) was calculated for each condition using pre and post body mass. %BML was higher for the CTRL (3.29 ± 0.68%) and HC conditions (3.08 ± 0.59%) than the HCF condition (1.60 ± 0.53%). (see Figure 3.1) MD, (95%CI), p-value, and ES indicate large differences between [HCF-HC]= -1.48% (-0.86 to -2.10), p=0.001, ES=2.64 and [HCF-CTRL]= -1.69% (-1.07 to -2.31), p=0.001, ES=2.77; but not for [HC-CTRL]= -0.21% (-0.83 to 0.41), p=1.000, ES=0.33, indicating that fluid replacement was successfully achieved in the HCF condition and it was in fact different than HC and CTRL. %BML POST was negatively associated with POST TNFα, and IL-10. (see Table 3.1)

Hydration Indices

PRE USG values for HCF, HC and CTRL were 1.014 ± 0.006, 1.017 ± 0.008, and 1.014 ± 0.006 while POST USG values were 1.016 ± 0.006, 1.017 ± 0.006, and 1.015 ± 0.007 for HCF, HC, and CTRL respectively. No main effect for time x condition ($F_{2,22}$= 0.767, p= 0.477, η^2 = 0.065, observed power= 0.164), condition ($F_{2,22}$= 0.076, p= 0.972, η^2 = 0.007, observed power 0.060), or time ($F_{2,22}$= 0.511, p= 0.490, η^2 = 0.004, observed power= 0.1000) were observed for urine specific gravity (USG). MD, (95%CI), p-values, and ES at POST indicate [HCF-HC]= -0.007 (-0.007 to 0.006), p= 1.000, ES= 0.17; [HCF-CTRL]= 0.013 (-0.005 to 0.007), p= 1.000, ES= 0.15; and [HC-CTRL]= 0.002 (-0.004 to 0.008), p= 1.000, ES= 0.30 indicating that hydration as assessed by specific gravity was very similar between conditions. PRE and POST USG is depicted in Figure 3.2

Rectal Temperature

At baseline rectal temperatures were not significantly different at PRE ($F_{2,33}$= 0.89, p=0.915). At baseline HCF, HC and CTRL were 37.22 ± 0.28℃, 37.17 ± 0.35℃, and 37.18 ± 0.29℃ respectively. A significant time x condition interaction was observed ($F_{38,418}$= 2.674, p=0.049, η^2 = 0.196, observed power= 1.000). Independent of time, we observed differences between conditions for T_{RE} ($F_{2,22}$ = 6.482, p=0.018, η^2 = 0.371, observed power= 0.721). Independent of condition, we observed differences between time points for T_{RE} ($F_{19,209}$ =166.110, p=0.001, η^2 = 0.938; observed power =1.000). T_{RE} of the HCF group was different than CTRL condition starting at minute 66 (p=0.026) and continuing through the POST time point (p= 0.005). (Figure 3.3) Upon exiting the environmental chamber POST T_{RE} was 38.64 ± 0.39℃, 38.86 ± 0.45℃, and 39.24 ± 0.45℃ for HCF, HC and CTRL respectively. Differences in T_{RE} POST were positively associated with POST IL-6, %BML, and CORT. (see Table 3.1)

Heart Rate

No significant differences in HR between conditions at any time point ($p>0.05$). Analysis of time x condition revealed no interaction ($F_{14,154}$=6.087, p=0.143, η^2 = 0.148, observed power= 0.465. Independently of time, we observed differences between conditions for heart rate (HR) ($F_{2,22}$ = 4.552, p=0.044; η^2 = 0.293; observed power= 0.563). Independent of condition, we observed differences between time points for HR ($F_{7,77}$ =232.836, p=0.001, η^2 = 0.955, observed power= 1.000). At minute 57, 72, 87 of the treadmill walking protocol, HR [MD (95%CI), p, ES] for [HCF-CTRL] were -8.0 b·min^{-1} (-23.0 to 6.0), p= 0.458, ES= 0.58; -13.0 b·min^{-1} (-27.0 to 2.0), p= 0.099, ES=0.88; -10.0 b·min^{-1} (-26.0 to 5.0), p= 0.325, ES=0.72; respectively. (See Figure 3.4) HR POST was positively associated with IL-10, IL-6 and IL-8.

Testosterone

Independent of group a significant main effect of time was observed for Testosterone ($F_{1,10}$= 38.53, p= 0.001, η^2= 0.794, observed power= 1.000). (see Figure 3.5) No significant group ($F_{1,10}$= 1.542, p= 0.238, η^2= 0.134, observed power= 0.289) or time x condition interaction ($F_{2,20}$= 0.178 , p= 0.838, η^2= 0.018, observed power= 0.074) was observed. Between conditions testosterone MD, 95%CI, and ES values were minimal (see Table 3.2 and 3.3) however percent change from PRE to POST for T were 33 ± 29%, 36 ± 21% and 33 ± 26% for

HCF, HC and CTRL respectively and demonstrated moderate effect sizes (0.53, 0.63 and 0.56). (see Table 3.4) T POST was significantly positively associated with IL-12p70 and negatively associated with IL-6. (Table 3.1)

Cortisol

Cortisol for the HCF, HC and CTRL conditions increased 3 ± 16%, 14 ± 25%, and 15 ± 21% however no significant interaction for time x condition was observed ($F_{2,22}$= 2.315, p= 0.122, η^2= 0.174, observed power= 0.419). Moderate and large effects were observed from PRE to POST for HC and CTRL but not for HCF. (see Figure 3.6) Independent of group, time appeared to be trending towards significance ($F_{1,11}$= 4.515, p= 0.057, η^2= 0.291, observed power 0.491) and effect sizes were moderate (HC= 0.62) and large (CTRL= 0.83) indicating that cortisol was increasing in these conditions more than HCF (ES=0.12). (see Table 3.2) Furthermore when examining the MD between conditions [HCF-HC] and [HCF-CTRL] were -10% (-32 to 11), ES=0.52 and -11% (-33 to 11), ES=0.59, respectively. (see Table 3.3)

Creatine Kinase

Independent of group, a significant main effect for time was observed ($F_{1,11}$= 39.818, p= 0.001, η^2= 0.784, observed power= 1.000). (see Figure 3.7) All conditions increased in % change from PRE to POST (34 ± 19%, ES= 0.67, 44 ± 31%, ES= 0.55, and 38 ± 43%, ES= 0.38) for HCF, HC and CTRL. (see Table 3.4) No significant main effect for group ($F_{2,22}$= 0.541, p=0.590, η^2= 0.047, observed power= 0.128) or interaction ($F_{2,22}$= 0.561, p=0.578, η^2= 0.049, observed power= 0.131) was observed. Between groups, mean differences indicate little to no change. (see Table 3.3)

Interleukin-8

A significant interaction for condition x time was observed for IL-8 ($F_{2,20}$= 10.500, p= 0.001, η^2= 0.512, observed power= 0.974) in addition to a main effect for time ($F_{1,10}$= 83.739, p= 0.001, η^2= 0.893 observed power= 1.000). (see Figure 3.8) Raw concentrations for IL-8 are presented in Table 3.5. From PRE to POST, all conditions demonstrated large effects (0.99, 3.31, 2.48) for HCF, HC, and CTRL. (see Table 3.6) Although no significant differences between conditions at POST were observed, mean differences, 95%CI and ES were strong for [HCF-HC] -1.08, (-2.42 to 0.26), ES= -0.84. (see Table 3.7) . •IL-8 from PRE to POST revealed a significant difference between the HCF and the HC groups (p= 0.008) with a large mean difference between HCF and CTRL of -1.15pg/mL (-2.43 to 0.14), ES= -0.97. (see Figure 3.9)

Interleukin-6

Independent of group, IL-6 showed a significant main effect for time ($F_{1,10}$= 20.734, p=0.001, η^2= 0.675, observed power= 0.983) with all values increasing from PRE to POST with large effect sizes. The CTRL condition demonstrated the greatest increase in absolute concentration to 17.23 pg/mL ± 15.06 range (5.18pg/mL - 59.01 pg/mL) ES= 1.43 although no significant condition x time interaction was observed ($F_{2,20}$= 2.831, p= 0.083, η^2= 0.221, observed power= 0.493). (see Figure 3.10) Additionally, at POST the HCF condition was -5.84 pg/mL (-17.56 to 5.88), ES= 0.85 and -6.21pg/mL (-17.94 to 5.51), ES= 0.54 less than HC and CTRL. (Table 3.7) •IL-6 from PRE to POST revealed a mean difference between HCF and CTRL of -6.73pg/mL (-18.71 to 5.26), ES= -0.75. (see Figure 3.11)

Other Cytokines: TNFα , IL-10, IL-1β, IL-12p70

All other cytokines included in the analysis demonstrated no significant main effect for time nor any significant interaction for condition x time. IL-1β however demonstrated a moderate effect between [HCF-HC] at POST= -0.54pg/mL (-1.42 to 0.34), ES=-0.58. Many of the values for IL-12p70, TNFα, IL-1B, and IL10, were below the detectable limit of 17.29pg/mL, 19.41pg/mL, 16.1pg/mL, and 8.76pg/mL respectively. PRE and POST values for all cytokines are presented in Table 3.5 and mean differences between conditions are presented in Table 3.6.

DISCUSSION

Based on the results from this investigation the most important findings are that hand cooling with fluid resulted in lower body temperature, moderately lower circulating cortisol levels, and moderate to large reductions in the inflammatory response for IL-6 and IL-8 compared to HC and CTRL. HC alone did not appear to result in any significant reductions in stress compared to the CTRL condition and was in fact very similar.

Proper Hydration Was Achieved

Our participants in the HC and CTRL group lost > 3%BML which is not uncommon to sport such as American football especially during exercise in hot environmental conditions. The HCF condition only lost 1.6% of their body mass during the exercise session which is in accordance with the ACSM and NATA fluid position statement which recommend fluid replacement to minimize sweat losses >2%.[16,17] Participants in our study achieved this recommendation indicating that a substantial amount of fluid was successfully replaced. (see Figure 3.1)

Reduced Body Temperature With Hand Cooling and Fluid

Starting at minute 66 of exercise, T_{re} was significantly lower in the HCF group compared to the CTRL group ($p < 0.05$). (see Figure 3.3) At POST the HCF condition T_{re} was 0.6°C (1.08°F) lower than the CTRL condition. Furthermore, •T_{re} from PRE to POST for HC and CTRL was 1.70 ± 0.48°C and 2.06 ± 0.40°C while HCF demonstrated the smallest •T_{re} with only a 1.43 ± 0.35°C increase. T_{re} were not different between the HC and CTRL conditions immediately post-exercise or upon exiting the environmental chamber however HC was not different than HCF. This indicates that although hand cooling alone provided little benefit during exercise compared to the CTRL condition, it was not different than HCF. This led us to believe that there was some effect of HC only, otherwise HCF would have been different than HC as well. Interestingly, following the final 3 minute bout of hand cooling treatment from minute 87 to minute 90 the HC condition appeared to demonstrate a much more rapid response. During the performance tasks completed from minute 90 to the POST time point (exit from the heat chamber) T_{re} dropped 0.19°C in 30 minutes in the HC condition while the CTRL condition continued to increase another 0.09°C. (Figure 3.3)

It is well known that an exercise at higher temperatures reduces sweating and forearm blood flow and that an increased total blood volume through fluid replacement results in increased blood delivered to the periphery.[18–22] This increase in blood flow and its influence on hyperthermia was unknown until Montain and Coyle[18] demonstrated that fluid replacement attenuated hyperthermia by promoting increased skin blood flow and that forearm skin blood flow during exercise increased 16% and was 17-20% higher than no fluid. In the present study the reduction in thermal strain experienced by the HCF group appears to be primarily due to the increased fluid rather than the hand cooling unit. The heat removed from the fluid may have resulted in reduced body temperature as was observed by Cheung and McLellan[23] who observed a difference of 157.5 kJ and 90.8kJ of heat storage during light and heavy exercise between fluid and no fluid conditions.

Contrary to the theory that heat was removed by fluid alone, it is plausible that the fluid may have increased the efficacy of the hand cooling unit. One potential theory to explain why hand cooling was more effective with fluid is that the increase in total blood volume may have delivered more blood to the periphery[18] which was in turn cooled by the device and delivered back to the circulation.[24] To explain why the HC condition alone did not show the same reductions in T_{re} may be a result of the reduction in peripheral blood flow due to the large amount of dehydration or the possibility that some participants may have been non-responders to the cooling intervention. This reduction in blood flow may have reduced the potential to cool.

Alternatively, the other theory explaining the lower T_{re} of the HCF condition is that heat may have been absorbed by the water [17.22℃ (63.0℉)] in the digestive tract that was replaced during the trial. Studies have shown that body temperature can successfully be reduced through the administration of fluid and cold ice slurries.[25–27] One limitation of this trail is that unfortunately no fluid alone trial was conducted in this experiment to further elucidate these findings. The addition of this trial would help isolate the influence of the water separate from the HCF condition.

Cortisol appeared to respond to the differences in $T_{re.}$ The smallest %change (3 ± 16%) from PRE to POST for CORT was observed in the HCF group while a moderate (ES= 0.62) and large (ES= 0.83) increase in the stress response occurred for HC and CTRL respectively. Taking a closer look at the •% change comparison between HCF and the other two groups, we saw very similar results to the differences observed in body temperature. These results suggest that the reduction in hyperthermic stress that occurred through hand cooling and fluid replacement led to moderate reductions (ES range= 0.52-0.59) in circulating markers of cortisol occur.

Cortisol, IL-6 and IL-8 Responses:

It has been well documented that cortisol is a strong anti-inflammatory hormone and its role in the inhibition of cytokine synthesis and activation of anti-inflammatory cytokines during exercise stress is critical to appropriate response to stress.[28–31] In order for proper homeostasis to occur it would appear that exercise stress induced secretion of cortisol promotes the release of factors that inhibit the release of cytokines to prevent systemic inflammation from occurring. IL-6 which serves as both a pro- and anti-inflammatory cytokine, will often increase within the first hour of prolonged exercise, during short-intensity exercise, during heat stress and resistance exercise. This suggests that IL-6 is very sensitive to all types of stress. The association of the cortisol response to rectal temperature observed in this study (r= 0.415; p<0.001) (see Table 3.1) was similar to Rhind et al.[12] who observed a significant correlation (r= 0.354; p<0.05). Our results were able to elicit significant changes in IL-6 POST exercise with uncompensable heat stress even though exercise intensity was relatively low.

IL-6 cytokine production is very closely linked to the increase of body temperature[3,6–9,12,32,33] and has been shown to increase with exercise alone,[8,34] exercise in the heat,[12,32,33,35] and a passive hyperthermic environment.[36] We observed a significant association between IL-6 and T_{re} (r= 0.415; p<0.001) (see Table 3.1) Unlike Rhind et al.[12] who observed only an association between T_{re} and TNFα (r=0.493; p<0.001). The inherent difference between our studies was individuals were in an uncompensable heat stress environment which elevated body temperature rather quickly. This resulted in rather quick elevations in IL-6 which often seen in EHS patients.[37–39]

IL-8 is a chemokine that is well known to activate basophils, eosinophils and T-lymphocytes.[40] Similar to IL-6, IL-8 has been observed to change during EHS,[3,10] exercise[34,41] and passive hyperthermia.[36] Furthermore, it has been shown to increase with both short-term maximal exericse[41] as well as prolonged endurance exercise[42] which suggests that hyperthermia, duration and intensity all influence IL-8 release. In our study, IL-8 increased significantly due to the hyperthermic exercise conditions regardless of trial and although the exercise intensity was relatively low, the stress from the inability to dissipate heat efficiently, adequately challenged the bodies ability to thermoregulate. Although we observed a significant interaction between the groups over time, post hoc tests did not reveal a significant difference between the groups at PRE, POST. Examining the raw PRE to POST values for IL-8, a large effect (ES=0.99), compared to the very large effects observed for HC and CTRL (ES=3.31, 2.48) indicating that IL-8 changes were less due to the reduction in the level of hyperthermia. • IL-8 levels however revealed a significant increase for the HC group compared to the HCF group.

In the current research model, hand cooling with fluid successfully influenced body temperature and moderate to largely (ES= 0.58-0.85) altered IL-6, IL-8, IL-1β between the HCF and other two conditions. The response observed confirms other studies depicting that acute physical stress both maximal and sub-maximal prompt the inflammatory cascade with release of other cytokines.[6,9,33,43] Furthermore this study aligns with the responses observed during exercise in the heat.[6,8,38] The IL-10, TNFα, and IL-12p70 responses observed in this study were very low and often below the detectable limit.

Human exercise during uncompensable heat stress with hand cooling and fluid may be a model for others examining cooling during exercise to follow. Although cytokines were not significantly altered, large effects were seen while wearing and American Football uniform during a relatively short exercise duration

(120minutes) at walking pace (range 1.56-2.01m/s [3.5-4.5mph]). This acute heat stress protocol was much shorter in duration and less intense than other research during marathons[6–8] however it reached similar levels of hyperthermia[33,35,32] and elicited large changes in the stress hormone cortisol and smaller increases when body temperature was reduced with cooling and fluid. Although our cytokine responses are not as large as those observed in EHS patients,[3,10,38,39,37] these results may suggest that lower levels of exertional hyperthermia with much less extensive severe levels of splanchnic blood flow and gut ischemia, may still prompt the release of the endotoxin lipopolysaccharide into the circulation.

CONCLUSIONS:

The results from this study demonstrate that hand cooling with fluid successfully decreased body temperature during uncompensable heat stress. Furthermore, we demonstrated that when body temperature was reduced moderate to large reductions in IL-6 and IL-8 were present which may suggest a decrease in the inflammatory response. Reduced body temperature with peripheral cooling and fluid replacement appeared to be vital in the decreased production of cortisol although creatine kinase and testosterone appeared to remain unchanged. Clinically, this investigation provides sports medicine personnel with evidence that when successful reduction in body temperature is achieved via cooling during exercise, there is a reduction in the stress and inflammatory response.

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Tables:

Table 3.1

Matrix of individual correlation coefficients (*r*) between the primary variables of measured

Variable	Tre	%BML	HR	IL-12p70	TNF-alpha	IL-10	IL-6	IL-1	IL-8	CORT	CK	T
Tre	1.000											
%BML	0.396*	1.000										
HR	0.226	-0.274	1.000									
IL-12p70	-0.062	-0.120	0.252	1.000								
TNF-alpha	-0.121	-0.394*	0.105	0.466**	1.000							
IL-10	0.064	-0.315*	0.379*	0.390*	0.507**	1.000						
IL-6	0.452**	0.100	0.489**	-0.143	0.047	0.335	1.000					
IL-1	-0.179	-0.048	-0.061	-0.053	0.331*	0.253	-0.015	1.000				
IL-8	0.174	0.049	0.500**	0.374*	0.443**	0.528**	0.519**	0.225	1.000			
CORT	0.415**	0.261	0.236	-0.087	-0.028	-0.048	0.458**	-0.065	0.341*	1.000		
CK	0.230	0.013	0.244	0.084	0.054	-0.027	0.091	-0.123	0.189	0.303*	1.000	
T	-0.148	0.136	-0.109	0.576**	0.028	-0.141	-0.444**	-0.085	-0.041	0.097	-0.016	1.000

Linear regression analysis of pooled data (HCF, HC, and CON groups) showing the correlation between continuous variables; *p< 0.05; **p<0.001

Tre= rectal temperature; %BML= % body mass loss; HR= heart rate; CORT= Cortisol; CK= Creatine Kinase; T= testosterone

TNF-alpha= Tumor necrosis factor alpha; IL= Interleukin

Table 3.2

Markers of Stress Response			
Pre Exercise			
	Creatine Kinase (U/L)	**Cortisol (nmol/L)**	**Testosterone (nmol/L)**
HCF	58.0±24.5	553.6±96.0	16.7±8.5
HC	54.3±33.4	549.2±106.7	15.1±6.8
Ctrl	66.0±47.6	558.8±87.0	16.0±7.5
Post Exercise			
	Creatine Kinase (U/L)	**Cortisol (nmol/L)**	**Testosterone (nmol/L)**
HCF	78.5±36.0	565.7±100.3	21.3±8.8
HC	74.7±40.4	612.2±95.1	20.2±9.1
Ctrl	83.1±53.6	628.9±81.4	20.4±8.0

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling

Table 3.3

Markers of Stress Response		Pre	Post	ES	% Change
Creatine Kinase (U/L)	**HCF**	58.0±24.5	78.5±36.0	0.67†	34±19
	HC	54.3±33.4	74.7±40.4	0.55†	44±31
	Ctrl	66.0±47.6	83.1±53.6	0.38	38±43
Cortisol (nmol/L)	**HCF**	553.6±96.0	565.7±100.3	0.12	3±16
	HC	549.2±106.7	612.2±95.1	0.62†	14±25
	Ctrl	558.8±87.0	628.9±81.4	0.83*	15±21
Testosterone (nmol/L)	**HCF**	16.7±8.5	21.3±8.8	0.53†	33±29
	HC	15.1±6.8	20.2±9.1	0.63†	36±21
	Ctrl	16.0±7.5	20.4±8.0	0.56†	33±26

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; ES= effect size (Cohen's d),†=moderate effect, *=large effect

Table 3.4

Markers of Stress Response: Mean Difference (95%CI) and Effect Size		Pre	ES	Post	ES
Creatine Kinase (U/L)	**HCF-HC**	7.2 (-30.7 to 45.1)	0.13	8.2 (-37.8 to 54.2)	0.10
	HC-Ctrl	-16.0 (-53.9 to 21.8)	-0.28	-12.8 (-58.8 to 33.2)	-0.18
	HCF-Ctrl	-8.8 (-46.7 to 29.0)	-0.21	-4.6 (-50.6 to 41.3)	-0.10
Cortisol (nmol/L)	**HCF-HC**	4.34 (-95.5 to 104.1)	0.04	-46.6 (-141.9 to 48.8)	-0.48
	HC-Ctrl	-9.6 (-109.3 to 90.2)	-0.09	-16.7 (-112.1 to 78.6)	-0.19
	HCF-Ctrl	-5.2 (-105.0 to 94.6)	-0.05	-63.3 (-158.6 to 32.1)	-0.69†
Testosterone (nmol/L)	**HCF-HC**	1.6 (-6.6 to 9.9)	0.12	1.0 (-8.3 to 10.4)	0.21
	HC-Ctrl	-0.9 (-9.1 to 7.4)	-0.02	-0.2 (-9.5 to 9.2)	-0.13
	HCF-Ctrl	0.8 (-7.5 to 9.0)	-0.11	0.9 (-8.5 to 10.2)	0.09

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; ES= effect size (Cohen's d),†=moderate effect, *=large effect

Table 3.5

Markers of Stress Response: Mean Difference for % Change

Variable	Condition	% Change	ES
Creatine Kinase (U/L)	**HCF-HC**	-10.0 (-43 to 23)	-0.39
	HC-Ctrl	5.4 (-28 to 39)	0.16
	HCF-Ctrl	-4.5 (-38 to 29)	-0.12
Cortisol (nmol/L)	**HCF-HC**	-10.8 (-32 to 11)	-0.52†
	HC-Ctrl	-0.2 (-22 to 21)	-0.00
	HCF-Ctrl	-11.1 (-33 to 11)	-0.59†
Testosterone (nmol/L)	**HCF-HC**	-2.6 (-30 to 25)	-0.10
	HC-Ctrl	3.0 (-25 to 31)	0.13
	HCF-Ctrl	0.4 (-27 to 28)	0.01

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; ES= effect size (Cohen's d),†=moderate effect, *=large effect

Table 3.6

Inflammatory Cytokine Responses By Condition: PRE and POST Exercise

	PRE Exercise (pg/mL)					
	IL-6	**IL-8**	**IL-10**	**IL-1B**	**IL-12p70**	**TNFa**
HCF	1.54±1.11	4.56±1.47	1.17±1.06	0.08±0.23	0.99±1.53	1.23±1.07
HC	1.60±0.90	3.88±0.86	1.44±1.10	0.69±1.93	0.91±0.99	1.09±1.20
Ctrl	1.92±1.53	4.03±0.90	1.49±1.06	0.23±0.68	1.03±1.11	1.41±1.75
	POST Exercise (pg/mL)					
	IL-6	**IL-8**	**IL-10**	**IL-1B**	**IL-12p70**	**TNFa**
HCF	11.02±6.03	6.01±1.47	1.61±1.39	0.08±0.16	1.24±1.96	1.33±1.72
HC	16.86±9.47	7.09±1.07	1.44±1.07	0.62±1.31	0.82±1.69	1.02±1.30
Ctrl	17.23±15.06	6.58±1.14	1.52±1.11	0.16±0.47	0.85±1.04	0.77±0.94

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; IL= Interleukin; TNFa= tumor necrosis factor alpha; *=significant difference

Table 3.7

Inflammatory Cytokine Response to Exercise from PRE to POST					
		Pre (pg/mL)	**Post (pg/mL)**	**ES**	**Fold Change**
	HCF	1.54±1.11	11.02±6.03	2.19*	7.89
IL-6	**HC**	1.60±0.90	16.86±9.47	2.27*	10.50
	Ctrl	1.92±1.53	17.23±15.06	1.43*	8.97
	HCF	4.56±1.47	6.01±1.47	0.99*	1.32
IL-8	**HC**	3.88±0.86	7.09±1.07	3.31*	1.83
	Ctrl	4.03±0.90	6.58±1.14	2.48*	1.63
	HCF	1.17±1.06	1.61±1.39	0.36	1.38
IL-10	**HC**	1.44±1.10	1.44±1.07	0.00	1.00
	Ctrl	1.49±1.06	1.52±1.11	0.03	1.20
	HCF	0.08±0.23	0.08±0.16	0.00	1.00
IL-1B	**HC**	0.69±1.93	0.62±1.31	0.04	-1.11
	Ctrl	0.23±0.68	0.16±0.47	0.12	-1.44
	HCF	0.99±1.53	1.24±1.96	0.14	1.25
IL-12p70	**HC**	0.91±0.99	0.82±1.69	0.06	-1.11
	Ctrl	1.03±1.11	0.85±1.04	0.17	-1.21
TNFa	**HCF**	1.23±1.07	1.33±1.72	0.07	1.09
	HC	1.09±1.20	1.02±1.30	0.06	-1.07
	Ctrl	1.41±1.75	0.77±0.94	0.45	1.83

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; ES= effect size (Cohen's d),†=moderate effect, *=large effect; IL= Interleukin; TNFa= tumor necrosis factor alpha

Table 3.8

Inflammatory Cytokine Mean Difference (95%CI) and Effect Size Between Conditions

Variable	Condition	Pre	ES	Post	ES
	HCF-HC	-0.06 (-1.37 to 1.24)	-0.06	-5.84 (-17.56 to 5.88)	-0.85*
IL-6	**HC-Ctrl**	-0.31 (-1.61 to 0.99)	-0.25	-0.37 (-12.10 to 11.35)	-0.03
	HCF-Ctrl	-0.38 (-1.68 to 0.93)	-0.28	-6.21 (-17.94 to 5.51)	-0.54†
	HCF-HC	0.57 (-0.63 to 1.78)	0.56	-1.08 (-2.42 to 0.26)	-0.84*
IL-8	**HC-Ctrl**	-0.15 (-1.35 to 1.06)	-0.17	0.51 (-0.83 to 1.86)	0.46
	HCF-Ctrl	0.43 (-0.78 to 1.63)	0.43	-0.57 (-1.91 to 0.76)	-0.43
	HCF-HC	-0.27 (-1.43 to 0.89)	-0.25	0.17 (-1.13 to 1.46)	0.14
IL-10	**HC-Ctrl**	-0.05 (-1.21 to 1.11)	-0.05	-0.83 (-1.14 to 1.21)	-0.07
	HCF-Ctrl	-0.32 (-1.48 to 0.84)	-0.3	0.84 (-1.21 to 1.38)	0.07
	HCF-HC	-0.62 (-1.90 to 0.67)	-0.44	-0.54 (-1.42 to 0.34)	-0.58†
IL-1B	**HC-Ctrl**	0.46 (-0.82 to 1.75)	0.32	0.46 (-0.42 to 1.34)	0.47
	HCF-Ctrl	-0.15 (-1.44 to 1.13)	-0.3	-0.08 (-0.96 to 0.80)	-0.23
	HCF-HC	0.09 (-1.24 to 1.43)	0.06	0.42 (-1.32 to 2.16)	0.22
IL-12p70	**HC-Ctrl**	-0.13 (-1.46 to 1.21)	-0.11	-0.30 (-1.77 to 1.71)	-0.21
	HCF-Ctrl	-0.03 (-1.37 to 1.30)	-0.03	0.39 (-1.35 to 2.13)	0.25
TNFa	**HCF-HC**	0.14 (-1.34 to 1.62)	0.12	0.32 (-1.15 to 1.79)	0.20
	HC-Ctrl	-0.32 (-1.80 to 1.16)	-0.21	0.25 (-1.22 to 1.72)	0.22
	HCF-Ctrl	-0.18 (-1.66 to 1.30)	-0.12	0.57 (-0.90 to 2.04)	0.40

HCF= Hand Cooling+ Fluid; HC=Hand Cooling; Ctrl= No cooling; ES= effect size (Cohen's d),†=moderate effect, *=large effect; IL= Interleukin; TNFa= tumor necrosis factor alpha

Figures:

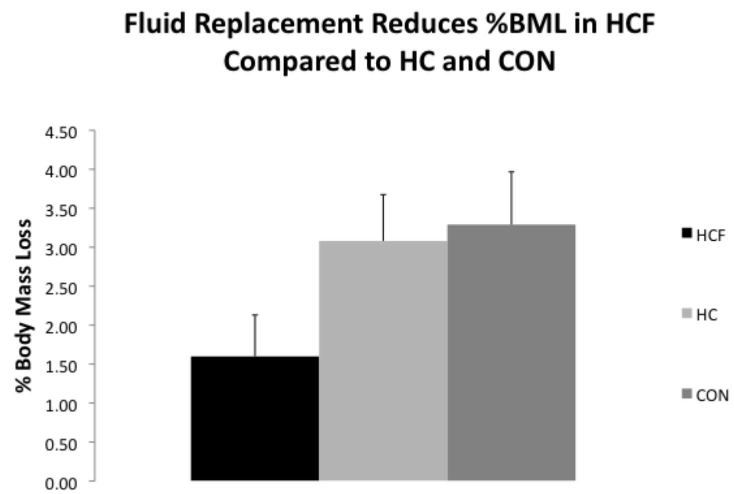


Figure 3.1 HCF=Hand Cooling + Fluid; HC= Hand Cooling; CON= No cooling

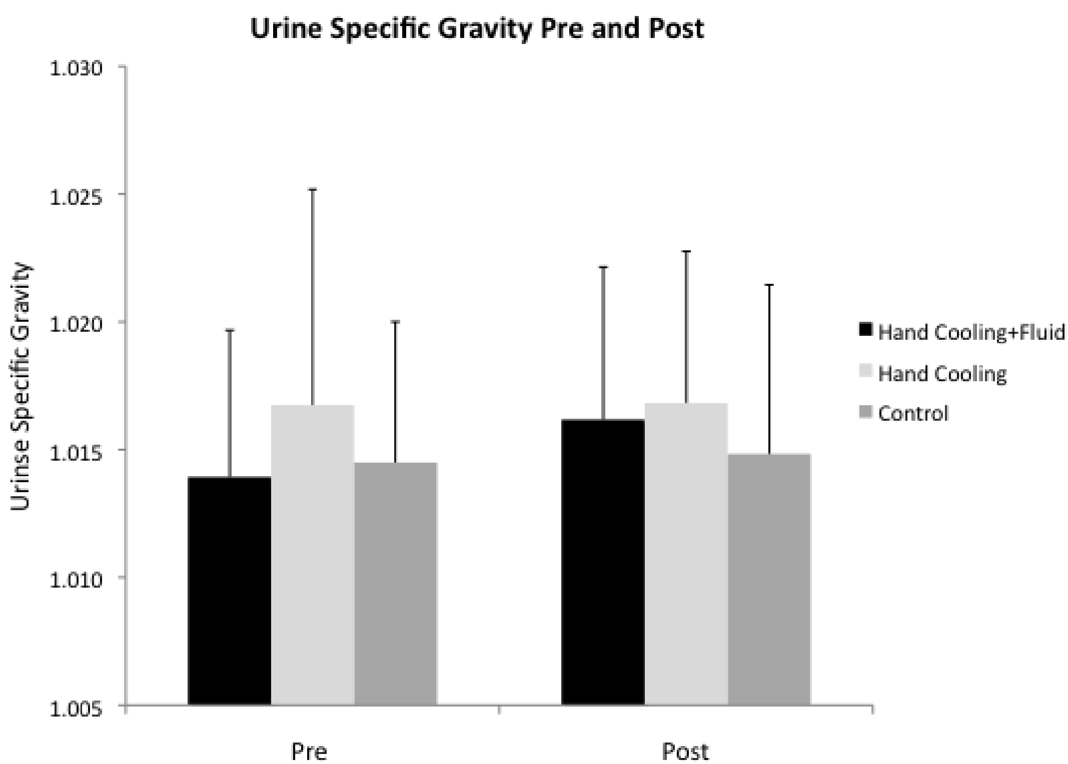


Figure 3.2

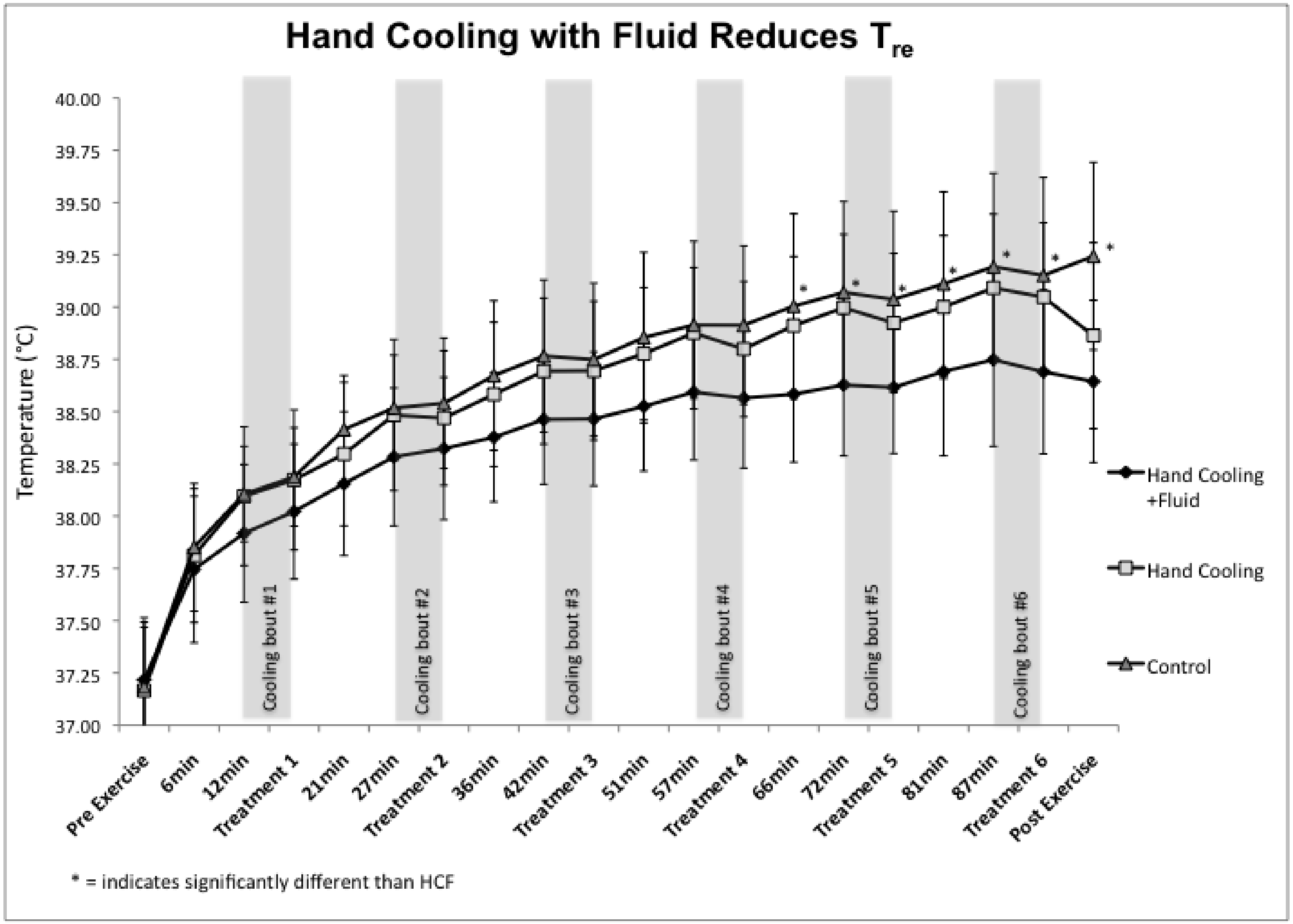

Hand Cooling with Fluid Reduces T_{re}
Temperature (°C)
40.00
39.75
39.50
39.25
39.00
38.75
38.50
38.25
38.00
37.75
37.50
37.25
37.00
Cooling bout #1
Cooling bout #2
Cooling bout #3
Cooling bout #4
Cooling bout #5
Cooling bout #6
Pre Exercise
6min
12min
Treatment 1
21min
27min
Treatment 2
36min
42min
Treatment 3
51min
57min
Treatment 4
66min
72min
Treatment 5
81min
87min
Treatment 6
Post Exercise
Hand Cooling +Fluid
Hand Cooling
Control
* = indicates significantly different than HCF

Figure 3.3

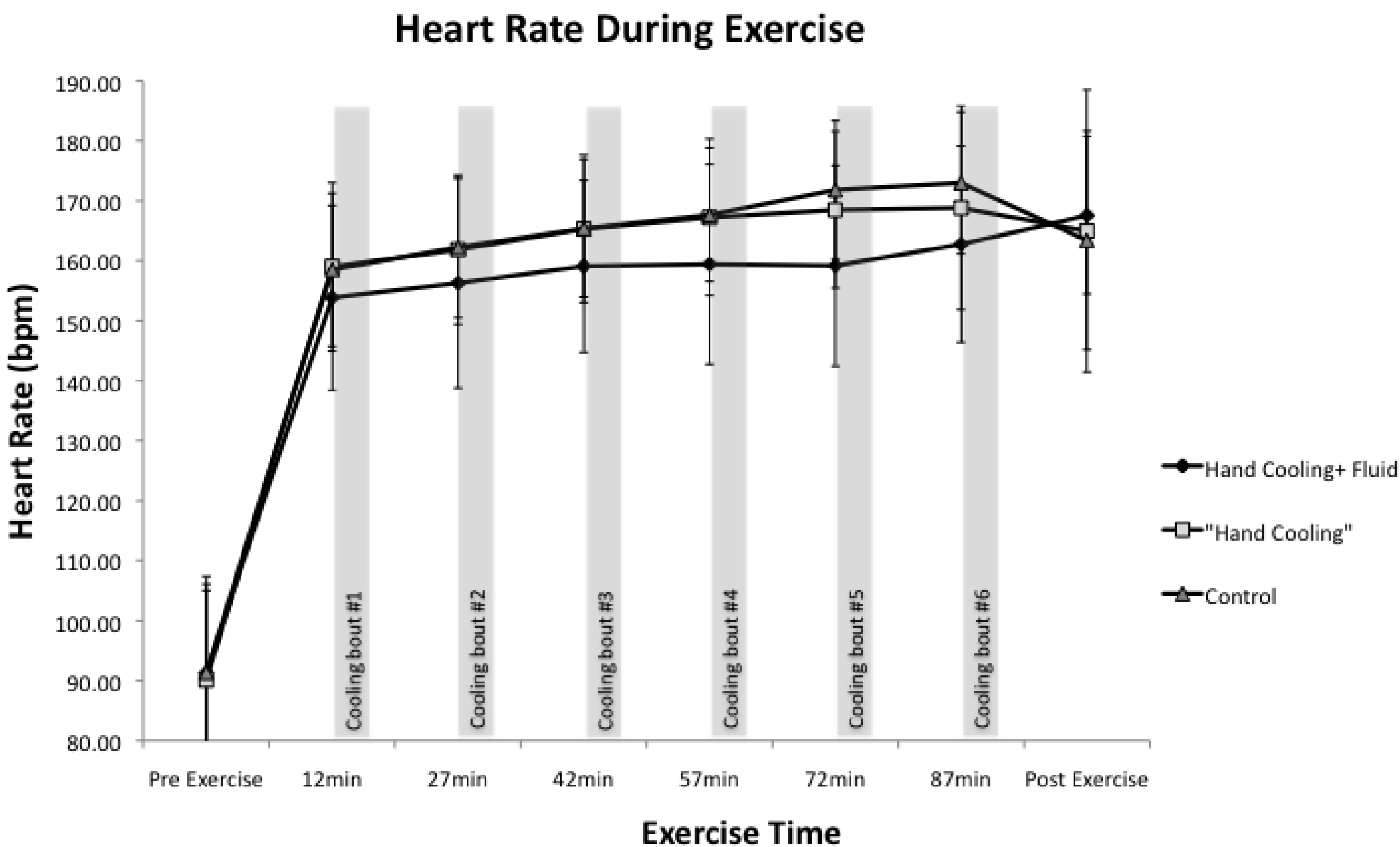
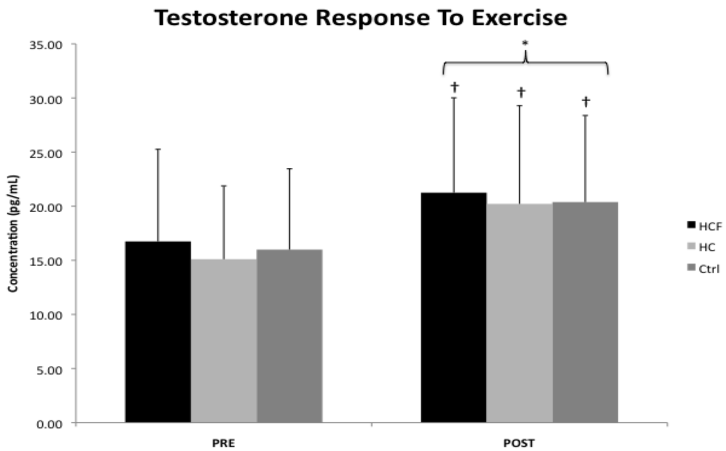
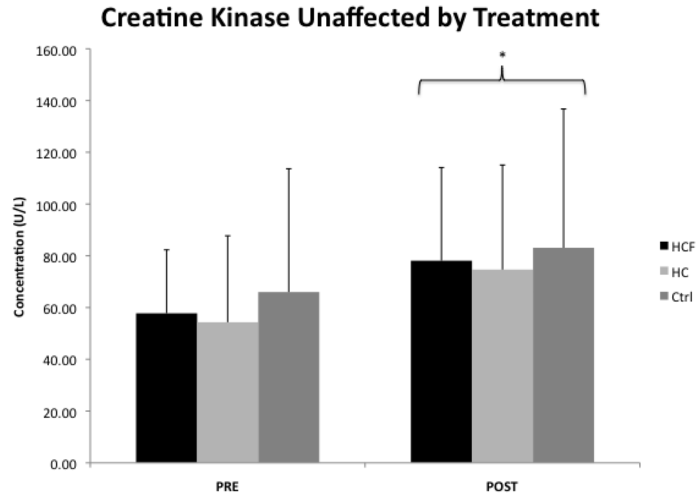

Heart Rate During Exercise
Heart Rate (bpm)
190.00
180.00
170.00
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
Cooling bout #1
Cooling bout #2
Cooling bout #3
Cooling bout #4
Cooling bout #5
Cooling bout #6
Pre Exercise
12min
27min
42min
57min
72min
87min
Post Exercise
Exercise Time
Hand Cooling+ Fluid
"Hand Cooling"
Control

Figure 3.4



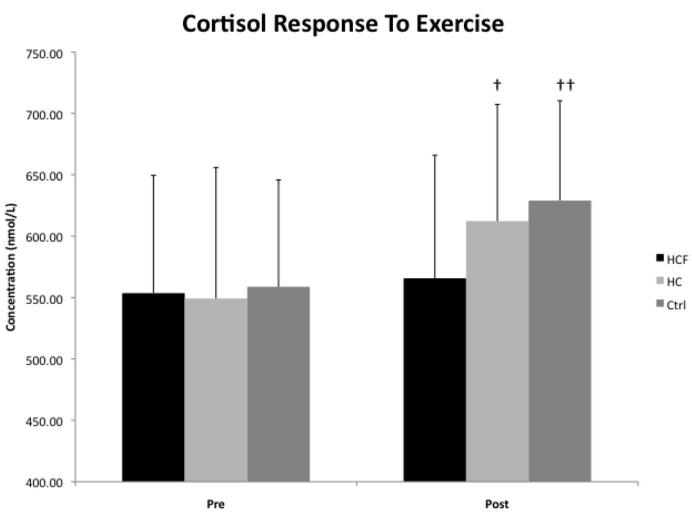
HCF= Hand Cooling+Fluid; HC= Hand Cooling; CTRL= No cooling
*= significant main effect for time, independent of condition;
†=Moderate effect size from PRE to POST

Figure 3.5



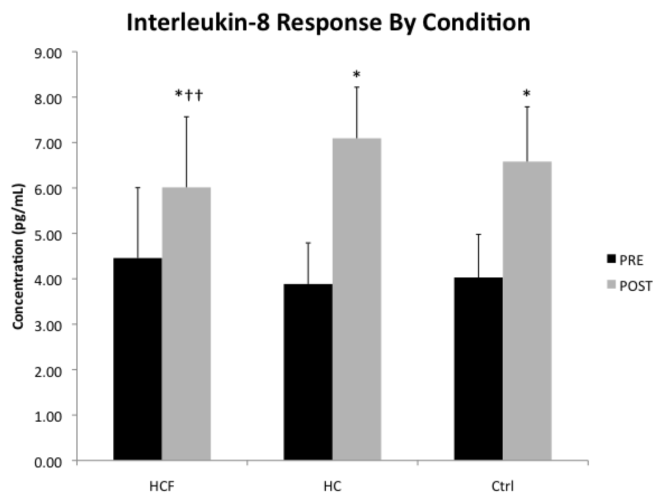
HCF= Hand Cooling+Fluid; HC= Hand Cooling; CTRL= No cooling
* Significant main effect for time, independent of condition

Figure 3.7



HCF= Hand Cooling+Fluid; HC= Hand Cooling; CTRL= No cooling
†= Moderate Effect size (0.67); ††= Large effect size (0.83) from PRE to POST

Figure 3.6



HCF= Hand Cooling+Fluid; HC= Hand Cooling; CTRL= No cooling
*= significantly different than PRE
††=Large effect size compared to CON

Figure 3.8

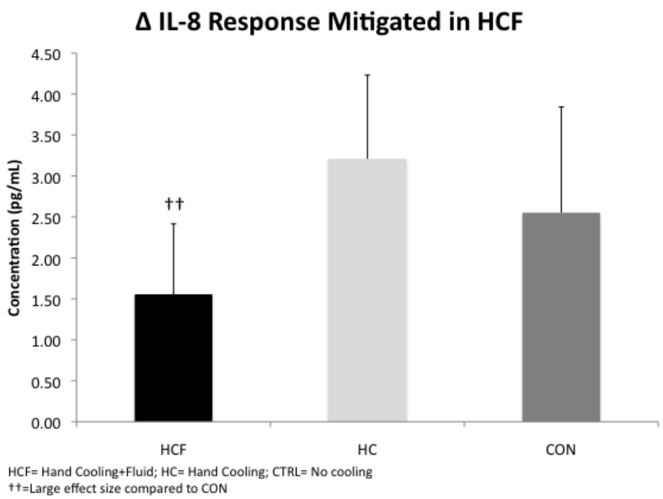


Figure 3.9

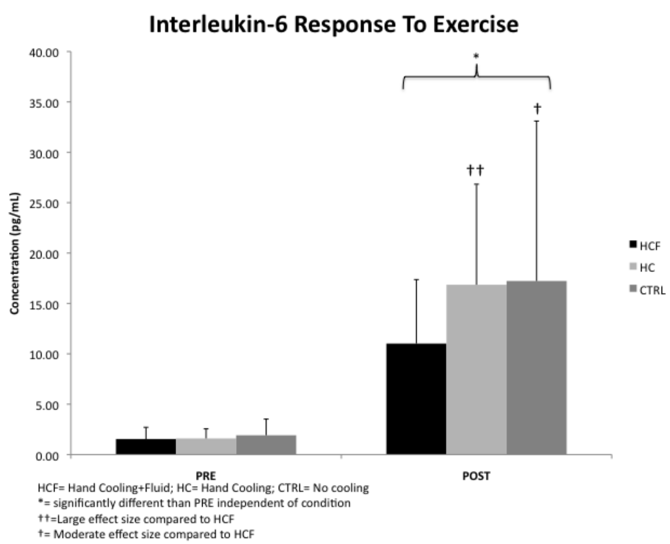


Figure 3.10

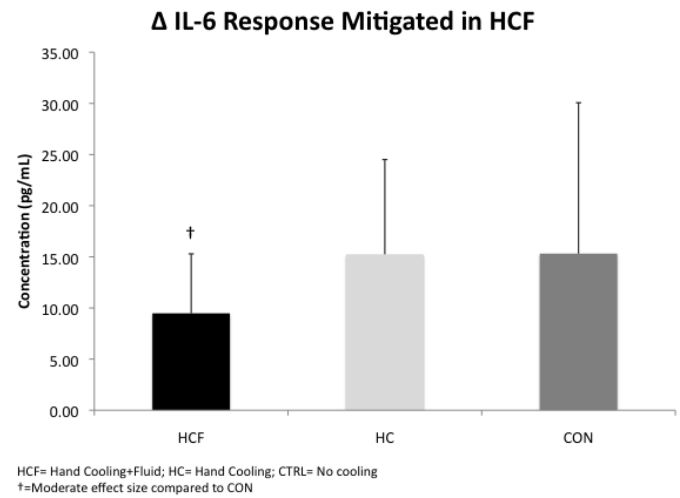


Figure 3.11

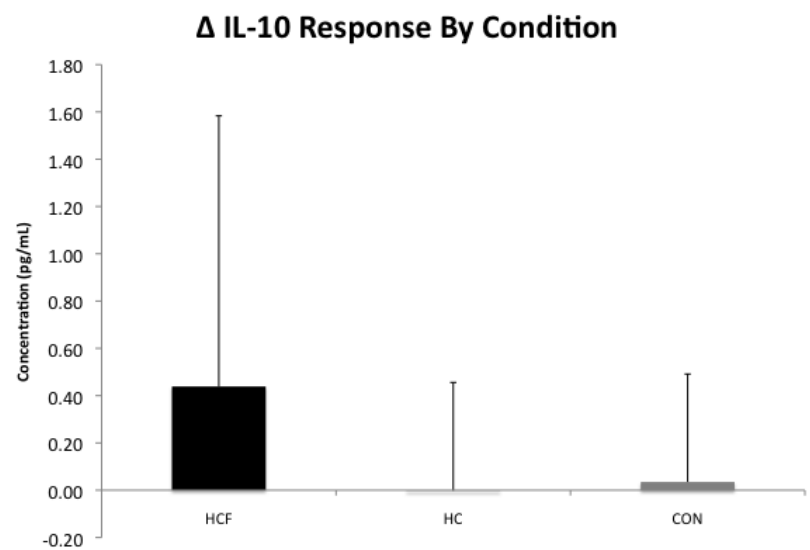


Figure 3.12

CHAPTER 4: THE INFLUENCE OF INTERMITTENT HAND COOLING ON BODY TEMPERATURE AND PERFORMANCE DURING AN AMERICAN FOOTBALL UNIFORM

ABSTRACT #1

Context: The American football uniform increases net heat storage and limits the evaporation required for thermoregulation. Maintaining hydration status and cooling during exercise have been shown to mitigate rises in core body temperature. Heat extraction via peripheral hand cooling coupled with fluid replacement may aid in reduced thermal strain. **Objective:** To determine the effect of intermittent hand cooling with and without fluid replacement on rectal temperature (T_{RE}). **Design:** Randomized crossover design. **Setting:** Research laboratory. **Participants:** Thirteen males (age: 24±3yrs, height: 179±5cm, body mass: 82.64±9.77kg). **Intervention:** Participants performed three separate 90- minute treadmill exercise bouts while wearing an American football uniform in a hot environment (39℃, 40%RH). Participants were randomly allocated to hand cooling (HC), HC with fluid replacement (HCF), and no cooling (CON). Participants performed hand cooling using a negative pressure device (~140mmHg) on 1 hand every 12th minute of exercise for 3 minutes in duration. **Main Outcome Measures:** Mean T_{RE} and heart rate (HR) were measured before exercise (PRE), during exercise every 12 minutes, and after exercise (POST). Body mass (BM), was collected PRE and POST. A repeated measures ANOVA for condition x time with post-hoc Bonferroni tests set at (• 0.05) were utilized to test between group differences. Mean differences, 95% confidence intervals, and effect sizes (MD, 95%CI, ES) were used to compare between conditions. Change in T_{REC} for every additional 1% body mass loss (BML) was also calculated. **Results:** %BML differed across conditions, (Mean±SD) HCF=1.60± 0.53%, HC=3.08±0.59% CON=3.29%±0.68% confirming that HCF partially replaced sweat losses compared to HC and CON (p=0.001, p=0.001)

respectively. A significant main effect for time was observed for T_{RE} in all conditions (p• 0.05). Starting at minute 57 and through POST, HCF T_{RE} was significantly lower than CON (p• 0.05). T_{RE} for HC was not statistically different than HCF or CON at any time point (p• 0.05). •T_{RE} for HCF, HC, and CON was 1.43±0.35℃, 1.70±0.48℃, and 2.06±0.40℃ respectively. •T_{RE} per 1%BML for [HCF-HC] and [HCF-CON] was -0.26±0.64℃ and -0.37±0.32℃ respectively. Moderate to strong effects for HR [HCF-CON] at min 57, 72, and 87 were MD= -8bpm, (95%CI= -22 to 6), ES=0.74; MD= -13bpm (95%CI= -27 to 2), ES=1.10; MD=-10, (95%CI= -26 to 5), ES=0.87 respectively. MD in HR for [HCF-HC] at min 57, 72, and 87 were MD= -8bpm, (95%CI= -22 to 6), ES=0.60; MD= -9bpm, (95%CI= -24 to 5), ES=0.71; MD= -6bpm, (95%CI= -22 to 10), ES=0.36. **<u>Conclusions:</u>** HCF significantly reduced T_{RE} starting at minute 60 while wearing an American football uniform. Furthermore, reductions in HR were observed in the HCF condition from minute 57 to 87. Increased blood volume in the HCF condition may have resulted in more effective heat transfer from the hand and reduced cardiovascular drift.

Word Count: 449

ABSTRACT #2

Context: Fluid replacement and cooling during exercise have been shown to improve the ability to thermoregulate during exercise in the heat. Wearing an American football uniform increases heat storage and presents a challenge to optimal cooling. New modalities such as peripheral cooling may reduce core body temperature and improve performance in the areas of power, speed, agility, reaction time, and balance.

Objective: To determine the effect of intermittent hand cooling with and without fluid replacement on sport-specific performance measures. **Design:** Randomized crossover design. **Setting:** Research laboratory. **Participants:** Thirteen males (age: 24±3yrs, height: 179±5cm, body mass: 82.6±9.8kg) performed three separate 90-minute treadmill exercise bouts in a hot environment (39℃, 40%RH) while wearing an American football uniform. **Intervention:** Participants were randomly allocated to hand cooling (HC), HC with fluid replacement (HCF), and no cooling (CON) in a counterbalanced order. Participants performed HC treatment using a negative pressure device (~140mmHg) on 1 hand every 12th minute of exercise for 3 minutes. **Main Outcome Measures:** Participants completed sprint speed on a non-motorized treadmill (Sprint), foot speed count (Count), counter movement vertical jump (VJ), reaction time (React), and modified balance error scoring system (BESS) performance battery before (PRE) and after (POST) exercise. T_{RE} was measured PRE and POST exercise. A repeated measure ANOVA for condition by time with post-hoc Bonferroni tests set at ($\alpha \leq 0.05$) were utilized to compare differences. Mean differences with 95% confidence intervals, effect sizes (MD, 95%CI, ES) and percent change in performance measures (%Δ) PRE to POST (%Δ, 95%CI, ES) were used to compare performance across conditions.

Results: POST T_{RE} for HCF (38.64±0.39℃) was significantly different than CON (39.24±0.49℃; p=0.005, ES= 0.61) but not HC (38.86±0.45℃; p=0.66 ES=0.25). POST T_{RE} for HC was not different than CON (p=0.111, ES=0.41). Sprint %Δ [HCF-CON] was 4.99%, (95%CI= -0.05 to 10.04), ES= 0.73, [HCF-HC] was 2.12%, (95%CI= -2.93 to 7.17), ES= 0.20, and [HC-CON] was 2.88%, (95%CI= -2.17 to 7.92), ES= 0.26. Count %Δ [HCF-CON] was 3.77%, (95%CI= -2.77 to 10.31), ES= 0.31, [HCF-HC] was 2.06%, (95%CI= -8.59 to 4.48), ES= 0.25, and [HC-CON] was 5.83%, (95%CI= -0.71 to 12.37), ES= 0.44. React %Δ for [HCF-CON] was -5.96%, (95%CI= -14.10 to 2.17), ES= 0.51, [HCF-HC] was -7.10%, (95%CI= -15.23 to 1.03), ES= 0.46, and [HC-CON] was 1.14%, (95%CI= -6.99 to 9.27), ES= 0.08. BESS Δ score [HCF-HC] was -21%, (95%CI= -53 to 12), ES= 0.46, [HC-CON] was -17%, (95%CI= -49 to 16), ES= 0.27, and [HCF-CON] was -37%, (95%CI= -70 to -5), ES= 0.53. **Conclusions:** HCF significantly reduced T_{REC} POST. Furthermore, HCF resulted in improvements in %Δ for Sprint and React compared to CON. Reduced thermal strain in the HCF condition may have allowed for a greater effort during performance tasks POST. **Word Count: 449**

INTRODUCTION

The ability to compensate or to maintain one's internal body temperature during exercise in the heat has been shown to be critical for performance. When the amount of heat gain equals the amount of heat loss the body is said to be in a compensable heat stress (CHS) situation however, when the heat gain exceeds the amount of heat loss, the body is in an uncompensable state. Uncompensable heat stress (UCHS) is defined as an environment where the heat stress index or HSI is equal to 1.0; (HSI = evaporation required divided by the evaporation max OR Ereq/Emax). When the HSI is equal to 1.0, there is a resultant net storage of heat in the body and therefore is unable to thermoregulate appropriately.

During uncompensable heat stress the evaporative heat loss required to maintain a steady state exceeds the maximal evaporative capacity of the environment and the body stores heat. In order to fully understand heat loss versus heat gain an understanding of the heat balance equation is essential.

Heat Balance Equation: $S=M-W\pm(E\pm R\pm C\pm K)$

- S= A positive S value represents the gain in heat storage by the body where a negative value represents net heat loss.
- M= the metabolic heat production and is determined by indirect calorimetry.
- W= the external work performed by the person
- R= the radiant heat exchange
- C= the conductive exchange
- K= the convective exchange
- E = the evaporative exchange which is divided into wet and dry
    - Dry is dependent on the temperature gradient
    - Wet loss is the evaporation of water or sweat.

Multiple research studies during UCHS have demonstrated that aerobic fitness, body fatness, and hydration have been influential factors in the rate of rise of internal body temperature and tolerance time. Interestingly, heat acclimation in a compensable environment has been the focus for many athletes, war fighters and laborers; however heat acclimation using short-term aerobic training has not been shown to aid in increasing tolerance during UCHS. This is opposed to the adaptations that we often see with individuals who are in CHS situations. During CHS, heat acclimation has been shown to increase the cardiovascular and thermal effects in a positive manner increasing exercise tolerance time and allowing the body to reach a state of thermal balance. This same method of heat acclimation prior to UCHS scenarios has been shown to have little influence on tolerance time and the increased sweat rate associated with heat acclimation caused dehydration to occur more quickly which can directly influence tolerance if fluids are not replaced. Many sporting environments are classified as compensable environments even during the summer months however when UCHS occurs the heat must be manually removed from the body.

Modalities focusing on heat removal such as vests, towels, fans, misters and even peripheral cooling have been developed to minimize the rate of heat storage. Recently, studies have shown tremendous insight into the use of peripheral hand cooling not only reduce core body temperature but to achieve an ergogenic effect in athletes during short and intense bouts of exercise.[2,36,37,49,50,76,78,79] The paradigm for this ergogenic effect is centered around core body temperature, and its effects on physiological functions.

As muscle activity increases during exercise there is an increase in internal heat storage. Muscle contraction breaks down ATP for energy, and the result is heat production. As intensity of exercise increases then muscle activation conversely increases causing more heat production. If the internal body heat production is greater than the heat dissipated via the body's thermodynamic system, then core body temperature will increase. This increase in core body temperature has been found to decrease whole body function and increase fatigue during exercise, so the goal of any cooling modality is to increase heat removal.[5,11]

Current research has shown that hand cooling modalities are a valid mode for removing heat from the body. However, it is less clear whether the attenuation of T_{CORE} leads to an increase in sport performance. Also, we have yet to find out if these performance gains can be accomplished while under uncompensable heat stress. By implementing a study with methodology based in clinical relevance to football athletes, we hope to better understand hand cooling's effect on foundational performance tasks (power, speed, reaction, balance) and the capabilities within a structured treatment duration.

At present, there is little to no data that investigates the effect a hand cooling device has on sport performance.[2,76,78] This study investigates the performance capabilities of individuals as they execute football specific tasks while in an uncompensable heat stress condition. Previous research has shown that performance decreases during heat stress, [3, 4,14,22,42,45,46,58] furthermore research examining the use of cooling modalities have shown that reducing body temperature during exercise in the heat allows for increased performance[1] and redistribution of blood flow to the exercising muscles rather than to the skin.[3,129] Therefore, the hypothesis of this study were that intermittent hand cooling will mitigate the rise of core body temperature and that intermittent hand cooling would increase performance during football specific tasks.

The primary objective of this protocol was to assess the influence of hand-cooling on core temperature (T_{CORE}) while wearing football equipment in a hot environment (temp+/- and humidity). A secondary aim was to examine the influence of hand cooling on football specific tasks.

METHODS

Participants:

Twelve healthy unacclimatized males participated in this research study. Percentage of body fat (%BF) and lean mass (LBM) was determined using a DEXA scanner (GE Lunar Prodigy Dual X-Ray Absorptiometry, General Electric Fairfield, CT) and software (GE Encore Healthcare 2006, Madison, Wisconsin) (Version 10.10.038). DEXA measurements were performed by a trained tech following standard procedures, according to the manufacturer's guidelines, while the participant was lying in a supine position. Participant demographics are presented in Table 2. This laboratory study was conducted in accordance with the University of Connecticut International Review Board (IRB). Participants completed informed and a self-administered medical history questionnaire prior to participation. Inclusion criteria were as follows: (a) 18-35 year old English speaking males (b) engaged in weight training or aerobic exercise 3 times per week or > 6 hours per week. The exclusion criteria included: (a) chronic health problems, (b) previous history of exertional heat stroke in the last 3 years, (c) history of cardiovascular, metabolic or respiratory disease, and (d) current musculoskeletal injury limiting physical activity. Participants' age in years (yrs), height in centimeters (cm), and mass in kilograms (kg).

Protocol:

Participants attended four sessions, one familiarization session and three testing sessions separated by a minimum of one week (Figure 20). The treatment order was randomized and counterbalanced. Participants executed the performance tasks as described followed by a 90 minute treadmill protocol with one of three treatment conditions, 1) Control (CON) 2) Hand cooling (HC) and 3) Hand cooling with fluid replacement (HCF). Physiological variables such as heart rate (HR), T_{CORE}, skin temperature (T_{skin}) as well as perceptual scales (RPE, Thermal, Thirst, Pain, Fatigue, ESQ) were recorded. Dependent variables specific to football performance included power using the countermovement vertical jump (CMJV) test, speed using a 6-second sprint (SPT) on a non-motorized treadmill, agility using both a fast feet drill (FF) and a reaction drill (REACT), and balance using the modified balance error scoring system (BESS).

Performance Battery

Participants were instructed on the correct procedures for the performance battery, which consisted of 5 specific tests aimed to examine power, agility, speed, and balance. Each performance task (CMVJ, BESS, FF/REACT drills, SPT) was performed until the participant was able to properly perform the task. This was done to minimize the learning bias during the testing trials and ensure subject safety.

Counter Movement Vertical Jump Task- The first performance assessment was a CMVJ using a non-conductive force plate (model 4060-NC; Bertec Corporation, Columbus, OH) controlled by Motion Monitor software (Innovative Sports Technology, Chicago, IL) during the balancing tasks. The force plate measured the vertical ground reaction force (VGRF) and collected kinetics at sampling frequencies of 1000 Hz during the performance of the CMVJ. This has been shown to be valid and reliable when assessing power in a CMVJ (ICC= 0.861).[112,113] The peak vertical ground reaction force will be normalized to body weight (N) for each participant (% body weight). Velocity and power were calculated from the VGRF data using the impulse-momentum theorem.[113]The CMVJ required participants to stand with both feet on the force plate remaining still while calibration took place. On cue the participant would step back with their dominate leg while simultaneously lowering their hips and swinging both arms backward. In one fluid motion participants would step back to the starting position with their dominate foot and drive arms forward as they jumped off both feet. They were instructed to leave and land with both feet on the force plate. Participants were asked to perform the CMVJ in duplicate, however, additional attempts were required if the task was performed incorrectly.

Modified Balance Error Scoring System Test- The modified BESS test was chosen to evaluate balance and postural control because of its high reliability and reproducibility (ICC =0.71; without DL stance).[117] The BESS test assessed the participant's balance with two different stances on two surfaces. The two stances included: Single Leg Stance (SLS), and Tandem Stance (TS). The SLS required participants to stand on one foot with their contralateral hip flexed 30 degrees and knee flexed 45 degrees. The TS required participants to stand with the heel of their dominate foot touching the toes of their non-dominate foot keeping both feet in line with each other. The two surfaces used were a firm, flat surface and a foam pad.

Participants were instructed to assume the standard testing position of eyes closed and hands on their hips and remain as still as possible for 20 seconds.

Participants were scored based on the number of errors they performed during the 20-second time period. Errors included: lifting hands off the iliac crest, opening the eyes, stepping, stumbling, or falling, moving the hip into more than 30 degrees of flexion or abduction, lifting the forefoot or heel, or remaining out of the testing position for more than 5 seconds. If a participant could not maintain the standard position for at least 5 seconds, the maximum score of 10 was given. Participants completed 2 attempts 2 for each SLS and TS on both firm and foam surfaces. Each BESS test was videotaped and exported where it was then scored separately by 2 certified athletic trainers familiar with grading of the BESS with a Kappa score of agreement=1.000.

Agility FF/ REACT Drill-

The two agility tests on the Quick Feet Board (The Quick Board LLC) (FF and REACT) were chosen because they are a reliable and accurate predictor of agility (ICC =0.89).[113] Both agility performance tasks required participants to utilize a feedback device connected to the Quick Feet Board. The board consisted of a mat positioned on the ground with the sensor pads in five locations (upper right and left, lower right and left, and center). A control device connected to the mat provided visual stimulus (five bright lights that correspond to the five foot pads) and feedback information as the movements were performed. Participants performed two attempts of the FF drill, which required them to perform the maximum number of foot touches during a 10-second interval. The maximum number of touches was recorded by The Quick Board and each attempt was separated by 30 seconds. Participants were then asked to perform two attempts of the REACT drill, which consisted of reacting to the visual stimulus on the control device. The numbers of correct and incorrect touches were counted during the

10 second trial and were recorded by the Quick Board Software (The Quick Board, LLC). So, we can assume that the measurements obtained on the Quick Feet Board would directly correlate to performance on the football field during drills requiring change in direction.[114]

6 second Non-motorized Treadmill Sprint-

The SPT task was performed on a Woodway Curve (The Curve, Woodway, USA, Waukesha, WI), non-motorized treadmill (NMT). With close replication of physiological workload and direct correlation to football play, the 6-s sprint test was an accurate indicator of football performance by utilizing maximum sprint speed (ICC = 0.941).[114] Prior to each sprint participants walked for 5 seconds, then ramped up their speed until maximum speed was obtained over a period of 10 seconds. Participants were asked to maintain the maximal effort for a minimum of 6 seconds and then decelerate to a slow walk for 45 seconds of active recovery between each sprint task. [114] Top speed was recorded for each 6- second sprint task using a hand held digital tachometer (Ametek USA, Largo, Florida).

Hand Cooling Device:

The hand cooling treatment device (Core Control, AVAcore Technologies, Ann Arbor, MI) utilized in this study consisted of a rigid chamber connected to a vacuum and water pump in which one hand could be inserted through an elastic structure forming an airtight seal around the forearm. The chamber is lined with bladders that surround the hand filled with cold circulating water maintained at (16.4^{o}C). Once the seal is created the device maintains a slight sub-atmospheric pressure (-40 mm Hg) through a vacuum pump. Both the circulating cold water and the suction are controlled through tubing secured to the chamber.

Familiarization Session:

Participants arrived in the same clothing and shoes they would wear under the equipment for each session. Each participant was properly fitted with a Football Helmet (Riddell; Speed), shoulder pads (Riddell; Power CPX 30), football pants (Nike; Team Apparel [with internal tailbone, Hip (Adams USA), Thigh (Bike) and Knee (Schutt) pads]) and football jersey (Nike; Team Apparel) over top of the shoulder pads.

Throughout the testing protocol participants were monitored via a rectal thermometer (YSI Spring Instruments, TX), heart rate monitor (Timex Group USA Middlebury, CT), and T_{skin} digital thermometers (Thermochron iButton +15°C thru +46°C Embedded Data Systems, KY USA). Participants were instructed to document their food and fluid intake and consume a similar diet and fluid volume in the 24 hours prior to each session. Upon arrival to the lab, participants provided a urine sample and inserted the calibrated flexible rectal thermometer 10 cm beyond the anal sphincter prior to beginning each session. Hydration status was confirmed using a hand held refractometer (Atago 300 CL, Atago, Japan) for urine specific gravity (Usg). Prior to each exercise session, all participants were required to begin exercise with a Usg of $\leq$ 1.020. Pre and post-exercise body mass was measured using a calibrated scale (model BWB-800A; Tanita Corp, Tokyo, Japan) to the 0.01kg, in order to determine percent body mass loss (% BML) and to determine fluid needs for the HCF trial. T_{sk} was measured in six locations (chest, deltoid, thigh, calf, right and left hands) using the surface digital thermometers attached by 3.8 cm x 6 cm strips of tape (Leukotape

L,BSN Medical South Africa). Thermometer buttons were place specifically in the following locations: 1) half way between the coracoid process and the nipple 2) over the insertion of the deltoid muscle 3) half way between and in line with the anterior superior iliac spine (ASIS) and the superior pole of the patella 4)half way between and in line with the calcaneal tuberosity and the joint line of the knee, and 5-6) on left and right hands were placed on the dorsal surface, along the mid-shaft of the 3rd metacarpal.

Participants donned the football equipment and completed an abbreviated 30-minute exercise protocol, which consisted of walking on a treadmill (Precor, Woodinville, VA) at a speed (3.5-4.5mph) and incline (5%). Participants were instructed to walk at a pace that they could sustain for the full 90 minutes but at an intensity that was moderately hard. This was to ensure that their core body temperature were to rise in hopes of them finishing exercise at or near 40°C. This method takes into account individual variation in stride length and rate of metabolic heat production. The rate of rise in core temperature was calculated by taking T_{CORE} every 6 minutes. The speed of the treadmill was adjusted during the familiarization trial if need be to ensure that all subjects were going to achieve the desired T_{CORE} that the investigators were looking to achieve.

To familiarize subjects on use of the hand-cooling device and the timing of using the device during the exercise, subjects were shown the proper way to use the CoreControl device during the familiarization session. All participants placed their right hand into the hand-cooling device for a duration of 3 minutes to simulate one bout of cooling. All instructions for use were performed in accordance with the recommendations made from the manufacturer. During testing, one researcher was blinded to the treatment and left the room while a second researcher entered the room to administer the treatment. During the control condition the device was worn by the participants but remained in the "off" position. During all treatments the participants remained seated to ensure body positioning was consistent. Participants were familiarized with perceptual scales of thirst, thermal sensation, fatigue, pain, environmental symptoms questionnaire (ESQ) and ratings of perceived exertion (RPE) as used in previous studies.[66, 67, 68, 69,70,71,72]

Testing Session:

Upon arrival for the testing sessions, subjects provided a urine sample and inserted the rectal thermistor as instructed during the familiarization session. The HR strap, T_{skin} sensors, and standard football game attire were placed on the participants. Once in place, the participants entered the temperature controlled environmental chamber (minute 0; temperature 38.44± 0.11 ^{o}C, relative humidity 34.94± 0.31%) and equilibrated for 10 minutes to allow for equilibration. Baseline measures of T_{CORE}, heart rate, and perceptual scales were obtained followed by 2 correct attempts of the pre-performance battery tasks (CMVJ, BESS Test, FF drill, REACT Drill, SPT).

T_{CORE}, heart rate, and perceptual scales were obtained prior to commencement of the 90-minute treadmill protocol, every 12th minute of exercise a three minute treatment session occurred. T_{CORE}, HR, and perceptual scales were recorded just prior to the completion of each treatment bout. The participant would exit the treadmill and remain seated with the treated arm resting on the thigh in a relaxed position for the duration of the treatment. Immediately after treatment participants re-entered the treadmill and continued exercising, for HCF group participants were asked to consume a bolus of water equal to sweat rate divided equally between the 6 treadmill bouts. Following the 90-minute treadmill protocol, the performance tasks (CMVJ, BESS Test, FF drill, REACT Drill, SPT) were performed again. T_{CORE}, HR, and the perceptual scales were measured again immediately post-performance tasks. Participants then exited the chamber; a post-exercise nude body mass, urine sample were obtained and rectal probe was removed.

Data Reduction and Statistical Analysis:

All data were analyzed using SPSS version 21.0 (IBM Corporation, Champaign, IL, USA) with an *a-priori* level alpha level of 0.05. We performed separate (condition x time) repeated-measures ANOVA for physiological, perceptual and performance dependent variables. All repeated measures ANOVAs were corrected using a Bonferroni *post hoc* test to evaluate any significant differences. Furthermore mean differences with 95% confidence intervals and cohen's-d effect sizes were utilized to quantify the magnitude of the difference between the conditions. One way ANOVA with Bonferroni post hoc tests was utilized to examine Δ values (Post value minus Pre value) and %change scores were calculated to further quantify the difference between the conditions. CMVJ force plate data were then exported into a customized software program (MatLab, The Mathworks; Boston, MA) and filtered using a low-pass, second-order 10 Hz Butterworth filter. The errors for each BESS position were summed to result in a Total BESS score, as well as individual scores for each position.

RESULTS:

Body Mass and Urine Specific Gravity:

Independent of time, we observed differences between conditions for body mass ($F_{2,22}$=125.07, p=.001). Independent of condition, we observed differences between time points for body mass ($F_{1,11}$=32.84, p=.0001). In order to ensure accuracy across groups, percentage of body mass loss (%BML) was calculated for each condition using pre and post nude body mass. The POST body mass (mean ± SD) was higher for the Ctrl and HC conditions HC= 3.08 ± 0.59%, Ctrl= 3.29 ± 0.68% than the HCF condition 1.60 ± 0.53%, HC= 3.08 ± 0.59%, Ctrl= 3.29 ± 0.68%). Mean difference (MD) and 95% confidence intervals (95%CI) indicated meaningful differences between [HCF-HC] 1.48% (0.86 to 2.10) p=0.001 and [HCF-Ctrl] 1.69% (1.07 to 2.31) p=0.001. (Figure 21)

Independent of time, we observed differences between conditions for urine color ($F_{2,22}$=3.597, p=0.047). Independent of condition, we observed differences between time points for urine color ($F_{1,11}$=4.014, p=0.021). The PRE urine color MD (95%CI) for [HCF-HC] was -0.33 (-1.43 to 7.65) p = 1.000 while [HCF-Ctrl] was 0.25 (-0.85 to 1.35) and [HC-Ctrl] was 0.58 (-0.52 to 1.68) p = 0.57.

No significant interactions were observed over time points or between conditions for Urine specific gravity (USG). There were no significant differences (mean ± SD) (p > 0.05) in PRE USG values between subjects in any of the conditions (HCF=1.01 ± 0.01, HC= 1.02 ± 0.01, Ctrl= 1.02 ± 0.01) or POST USG values (HCF= 1.016 ± 0.02, HC= 1.017 ± 0.02, Ctrl= 1.015 ± 0.02) (Figure 22).

Core Temperature:

We observed a significant interaction between time points and conditions for T_{CORE} ($F_{38,418}$= 2.674, p=0.049) (Figure 23). Independent of time, we observed differences between conditions for T_{CORE} ($F_{2,22}$ = 6.482, p=0.018). Independent of conditions, we observed differences between time points for T_{CORE} ($F_{19,209}$ =166.110, p=0.001). T_{CORE} was highest during the Ctrl condition starting at minute 66 compared to the HCF condition. (Figure 23) T_{CORE} was similar (p > 0.05) at baseline between groups (mean± SD) (HCF= 37.22 ± 0.28℃, HC= 37.17 ± 0.35℃, Ctrl= 37.18 ± 0.29℃). Upon exiting the environmental chamber POST T_{CORE} was HCF= 38.64 ± 0.39℃, HC= 38.86 ± 0.45℃, and Ctrl= 39.24 ± 0.45℃).

Mean differences, 95% confidence intervals and effect sizes between conditions for T_{CORE} are presented in Table 1. Both Table 3 and Figure 23 indicate that POST [HCF-Ctrl] was -0.60℃ (-1.04 to -0.16), p=0.024, ES=1.43, which began to diverge from Ctrl starting at minute 66 [HCF-Ctrl] -0.42℃ (-0.80 to -0.04), p=0.026, ES= 1.08. At POST the HCF group had a 0.6℃ (1.08℉) reduced body temperature compared to Ctrl. There were no significant mean differences in T_{CORE} between the HC vs. Ctrl conditions at any time point. Δ T_{CORE} PRE to POST was 1.43 ±0.35℃, 1.70 ±0.48℃ and 2.06 ±0.40℃ for HCF, HC and Ctrl respectively. Δ T_{CORE} MD (95%CI) from the PRE exercise time point to minute 87 (immediate post treadmill exercise) for [HCF-HC] and [HC-Ctrl] were -0.41℃ (-0.86 to 0.04), p=0.088, and -0.09℃ (-0.54 to 0.37) p= 1.00, while [HCF-Ctrl] observed a greater mean difference of -0.50℃ (-0.95 to -0.04) p = 0.028. Furthermore, when examining the mean difference (95%CI) in Δ T_{CORE} from the PRE exercise time point to the POST exercise time point just prior to leaving the chamber [HCF-HC] and [HC-Ctrl] were -0.27℃ (-0.69 to 0.15) p=0.347, ES= 0.65 and -0.36℃ (-0.78 to 0.06) p= 0.114, ES= 0.85 while [HCF-Ctrl] demonstrated a larger difference of -0.63℃ (-1.06 to -0.21)p = 0.002, ES=1.75.

The change in T_{CORE} for each additional 1% body mass loss ($\Delta\cdot 1\%BML^{-1}$) was calculated for the HCF trial compared to the HC and Ctrl groups. $\Delta\cdot 1\%BML^{-1}$ for [HCF-HC] was 0.18℃$\cdot 1\%^{-1}$ while [HCF-Ctrl] was 0.37℃$\cdot 1\%^{-1}$. If T_{CORE} is projected for each group the: a 5% BML in the HC group would result in a T_{CORE} of 40.86℃ (105.5℉), a 4% and 5% projected BML for the Ctrl group would cause a POST T_{CORE} of 40.68℃ (105.22℉) and 41.45℃ (106.61℉) respectively (Table 4).

The change in T_{CORE} for each of the six cooling treatments was determined and no significant differences (p>0.05) in T_{CORE} existed for any of the conditions. However, the overall the data trends toward an increase in heat removal during subsequent treatments. (Figure 24) A negative value designates a decrease in T_{CORE} from pre to post hand cooling and a positive value shows a continued increase in T_{CORE} during treatment.

Heart Rate:

Independently of time, we observed differences between conditions for heart rate (HR) ($F_{2,22}$ = 4.552, p=0.044. Independent of condition, we observed differences between time points for HR ($F_{7,77}$ =232.836, p=0.001. No significant differences in HR between conditions for any time point (p>0.05). There was a point at the 90 min mark during exercise that HR crossed as HCF increased while HC and Ctrl both decreased not at a significantly different level (p>0.05) (Figure 25). From minute 57-87 of the treadmill walking protocol, HR mean differences between [HCF-Ctrl] ($p>0.05$) were [MD (CI), ES] -8 bpm (-22.46, 5.96) 0.58, -13 bpm (-27.02, 1.68) 0.88, -10 bpm (-25.92, 5.42) 0.72, 4 bpm (-23.51, 15.18) 0.26 respectively (Table 5). The Δ HR for HCF, HC, Ctrl was 71 bpm, 79 bpm, 82 bpm respectively. When normalized to BML the $\Delta \cdot 1\%BML^{-1}$ for [HCF-HC] and [HCF-Ctrl] was -6.03 ± 10.31 bpm and -7.41± 8.14 bpm for every 1% respectively.

Performance Battery:

Balance Error Scoring System Task- We observed a significant interaction between time points and conditions for the total BESS score ($F_{2,22} = 3.761$, $p = 0.039$). BESS scores were highest during the Ctrl condition at POST compared with PRE. Furthermore, independent of condition, we observed differences between time points for the total BESS score ($F_{1,11} = 4.944$, $p = 0.048$) and for the SL FIRM errors ($F_{1,11} = 5.191$, $p = 0.044$. Independent of time point, we observed differences in errors between conditions during the SL FIRM stance ($F_{2,22} =3.512$, $p=0.047$). There were no significant differences ($p>0.05$) for Δ (PRE to POST) BESS scores between groups HCF, HC, Ctrl. The percentage change in errors within groups from (PRE to POST) for HCF, HC and Ctrl were 7.17±19.39% less errors, 13.31±28.28% more errors and 30.13±42.10% more errors respectively (Figure 26).

HCF, HC, Ctrl PRE exercise BESS had a mean of 14.42±5.30 errors, 13.75±6.06 errors, 15.00±6.33 errors respectively. POST BESS test scores for HCF, HC, Ctrl were 13.38±5.43 errors, 14.41±5.08 errors and 18.96±8.47 errors respectively. (Table 6) The percent change in errors between groups [HCF-HC], [HC-Ctrl], [HCF-Ctrl] PRE to

POST exercise was -20.50 % (-52.77 to 11.79); 0.84, -16.82% (-49.10 to 15.46); 0.47 and -37.31%(-69.60 to -5.03); 1.14 [% change (95%CI);ES] respectively (Table 8).

Countermovement Vertical Jump: Height and Power- During the CMVJ task, a significant main effect for time ($F_{1,11}$= 10.279, p=.008) was observed independent of condition. POST CMVJ height was significantly higher than the PRE values regardless of condition. No significant interactions (condition x time were observed) for jump height ($F_{2,22}$= 0.194, p=0.825) or for power ($F_{2,22}$= 1.232, p=0.294).

HCF, HC, Ctrl PRE exercise VGRF had a mean of 2618.52±956.45 N·m, 2809.95±429.35N·m, and 2864.24±507.88N·m respectively. POST VGRF for HCF, HC, and Ctrl was 2952.29±510.49 N·m, 2850.87±564.17 N·m, 2920.92±510.34 N·m respectively. (Table 6) The mean difference in percent change for VGRF between groups [HCF-HC], [HC-Ctrl], [HCF-Ctrl] was 3.07% (-3.87 to 10.01); ES=0.41, -1.07% (-8.93 to 4.94); ES=0.14 and 1.99%(-4.94 to 8.93); ES=0.40 [% change (95%CI);ES] respectively (Table 8).

HCF, HC, Ctrl PRE exercise jump height had a mean of 27.65±5.05cm, 28.00±4.17cm, and 28.79±6.35cm respectively. POST jump heights were increased in all groups (30.14±6.05cm, 30.05±6.57cm, 30.66±5.28cm) for HCF, HC and Ctrl respectively. The mean difference in percent change for jump height between groups [HCF-HC], [HC-Ctrl], [HCF-Ctrl] was 1.97% (-9.23 to 13.17); ES=0.18, -1.02% (-12.21 to 10.18); ES=0.08 and 0.95%(-10.25 to 12.15); ES=0.11 [% change (95%CI);ES] respectively (Table 8).

QuickBoard- Fast Feet drill- We did not observe any significant interactions between time points and conditions during the fast feet (FF) drill ($F_{2,22}$ =3.519, p = 0.074). There were no significant differences (p>0.05) between groups for average FF drill touches from PRE to POST. However, percent change PRE to POST shows a 1.48 ± 4.52% improvement for HCF group, 3.53 ± 5.61% improvements for HC group, and a 2.29 ± 8.31% decline in the Ctrl group (Figure 29). This translates into a percent change from PRE to POST of [% change (95%CI), ES]; 3.77%(-2.77, 10.31) 0.56, 5.83% (-0.71 to 12.37) 0.82 and -2.06% (-8.60, 4.48) 0.40 change for HCF-Ctrl, HC-Ctrl and HCF-HC respectively (Table 8).

There were significant differences (p≤ 0.008) within groups for attempt 2 PRE to POST (Figure 30). There were significant reductions in mean FF count between attempt 1 and attempt 2 for Pre and Post measures in the HCF, HC, and Ctrl trials, p-values 0.006, 0.007, 0.002 respectively, p-values for POST were 0.047, 0.001, and 0.000 respectively.

QuickBoard-REACT drill- We did not observe any significant interactions between time points and conditions during the REACT drill ($F_{2,22}$ = 2.733, p=0.087). There were no significant differences between groups (p>0.05) PRE to POST for reaction time(s)(Figures 33 and 34). However, the % difference between [HCF-HC], [HCF-Ctrl] and [HC-Ctrl] were 3.51%±6.55 faster, 3.60%±9.71 slower and 2.45%±7.06 slower respectively. The percent change in reaction time(s) from PRE to POST were [% change(CI), ES] 7.1% (-15.23 to 1.03), 0.86; 5.97% (-14.10 to 2.17), 0.88 and 1.14% (-6.99 to 9.27), 0.13 (Table 8). There were no significant differences (p>0.05) between groups for accuracy percentage during the REACT drill (Figure 31 and 32).

Non-Motorized Treadmill Sprint- We observed a significant interaction between time points and conditions for the SPT speed ($F_{2,22}$ = 3.499, p= 0.048) (Figure 36). PRE exercise NMT sprint had a mean of 15.77±0.71, 15.70±0.50, 16.07±0.57 for HCF, HC and Ctrl respectively (Table 6). However, POST exercise attempt 1 was significantly faster (p=0.006) than attempt 2 for the HCF group was whereas the HC and Ctrl groups showed no significant change in speed from attempt 1 to attempt 2 p-values=0.823 and 0.767 respectively. When the two attempts were averaged for pre and post sprint speed there were no significant changes (p>0.05) from pre to post between groups and mean differences between groups ([HCF-HC],[HCF-Ctrl],[HC-Ctrl]) with p-values of 0.892, 0.053 and 0.479 respectively. The percent change scores in average PRE and POST speeds indicate a 1.43%±2.40 improvement for the HCF group, a 0.68%±7.45 decrement in the HC group and a 3.56%±3.29 decrement in the Ctrl (Table 8).

DISCUSSION:

The purpose of this study was to investigate the effectiveness of the CoreControl™ hand cooling system as a treatment for reducing the associated symptoms on individuals exercising in a hot environment while wearing an American Football Uniform. A secondary aim was to examine if a reduction in heat stress via peripheral cooling of one hand would lead to an increase in football performance. In conjunction with previous studies[71,72,75-78,81] we observed an attenuation in the rise of T_{CORE}, reduction of HR, and subsequent improvements in performance particularly in the areas of balance, reaction, and sprint speed.

Core Body Temperature:

It has already been shown that continuous extraction of heat from the body through one hand during exercise is an effective mode of attenuating the rise in T_{CORE} and causes an increase in aerobic endurance[72] however, to our knowledge there has yet to be a study investigating the intermittent use of this device in an uncompensable heat stress situation while wearing a full American football uniform. Both the external heat stress (e.g. equipment and environmental conditions) and the internal heat stress (e.g. metabolic heat produced via exercise) produced during this experiment impaired the body's evaporative capacity, which is the primary thermoregulatory mechanism for heat loss. Previous studies[72,73,81] have reported a significant cooling effect while utilizing the hand cooling device when wearing recreational clothing in less severe environmental conditions. Furthermore, Grahn et al.[72] reported that effectiveness of the CoreControl™ decreased in an exponential manner with increasing exercise intensity indicating that there is a point where metabolic heat production (e.g. high intensity exercise) exceeds the ability to extract heat from the body. In our study, all groups received similar treatment and worked at similar intensities as these previously mentioned studies,[72,73,81] and while our HC group responded similarly to these past studies, the HCF treatment group responded more favorably to the hand cooling treatment. A potential explanation for this reduction in the POST HCF T_{CORE} response starting after the 66th minute of exercise (Figure 23) may have been due to the additional fluid received by the HCF group. It is theorized that the fluid replacement resulted in a concomitant increase in blood volume. This increased blood volume required by the exercising muscles to perform the activity as well as the skin to remove the heat via evaporation may have been a reason for the observed response. If this theory were true, this would provide further substantial evidence for the current body of research suggesting that fluid losses be minimized to <2.0% during exercise. In the current investigation the %BML of the HCF group of 1.5% was significantly less compared to the HC=3.08% (p=0.00) and Ctrl=3.29% (p=0.00). Unfortunately in this investigation we were unable to have a fluid only trial, however this research still suggests that the HC and fluid were additive in the reduction of T_{CORE} during UCHS.

Glabrous skin surfaces allow for the effective removal of heat due to the large volume of blood stored in AVA's and the close relationship to the skins surface.[63,96] Through the application of this external heat sink modality to the hand with fluid replacement, heat was removed via conduction from the body more effectively and thus mitigate the rise in T_{CORE} and HR.[63,75,76] Applying a hand cooling treatment does decrease the body's heat storage, and to gain a better understanding further examination of the heat balance equation is necessary. It is well documented that uncompensable heat stress implies that evaporative, convective, conductive and radiative mechanisms of heat loss are impeded. When a hand cooling modality is applied under these circumstances during uncompensable heat stress it is the only (-) value removing heat via conduction on the right side of the equation. Evaporation and convection are minimal due to the environmental temperature and humidity which is warmer and more saturated than the skin. Under these conditions minimal transfer is occurring. Furthermore the fluid replacement aided in the maintenance of blood volume which likely resulted in redistribution of blood to both the periphery and the exercising muscles.

To our knowledge this is the first study to investigate the additive influence of hand cooling with hydration on T_{CORE}. Intermittent removal of heat from the body when combined with hydration to minimize fluid losses during exercise can prevent an individual from approaching dangerous core temperature levels that may lead to a medical emergency such as EHS. With hydration policies and recommendations being followed by many athletes,[5,9] it stands to reason that understanding the additive effect hydration has on hand cooling is important for future research. The average mean difference (MD) in T_{CORE} between HCF vs. Ctrl from minute 66 through the POST time point was 0.46°C (0.83°F). This reduction in T_{CORE} is very important clinically when put into perspective. For example, when the change in T_{CORE} for each additional 1% body mass loss ($\Delta \cdot 1\%BML^{-1}$) is calculated out to 4 and 5% BML this would result in rates of increase that would be considered dangerously high for many medical professionals. (See Table 4)[16,31,32,118] Essentially if you took the same individual and provided them with HCF during one trial and the control treatment in another trial, for each additional 1% BML they would reach 40.68°C (levels equal to exertional heat stroke) at 4%BML compared to the HCF individual only at 39.2°C (normal exercising body temperature).

Cardiovascular Responses:

This investigation enabled the researchers to examine the relationship between T_{CORE} and CV strain. Since hand cooling treatments were delivered intermittently throughout exercise, we were able to examine the effect of HCF and HC on HR over time (Figure 25). As we expected the HCF group experienced a 10.0 $b \cdot min^{-1}$ reduction in HR compared to the HC and Ctrl groups for almost 30 minutes of exercise from minute 57-87. The reduction in heart rate is likely due to the fluid replacement, which during exercise in the heat has been well documented to reduce the effects of cardiovascular drift.[119] As a result of the fluid replacement, blood volume increase causing a subsequent increase in stroke volume which maintains a lower HR during exercise. HR in the HCF group was not significantly different, however upon the completion of exercise prior to performance tasks, an interaction was observed. The HC and Ctrl groups experienced a decrease in HR while the HCF groups HR experienced an increase (Figure 25). Those in the HCF group were able to give a greater effort at a higher intensity when asked to perform the agility, reaction, and sprint task specifically. For example in the SPT task a significant reduction ($p=0.006$) in speed from attempt 1 (16.57 ± 0.97) to attempt 2 (15.43 ± 0.83) POST was observed within the HCF group. This indicates that the reduction in HR from the HC and the fluid during the 90 minute exercise protocol enabled the HCF group to perform at a higher intensity and thus produced a faster speed than the HC and Ctrl groups. This is further substantiated by the equation for cardiac output $Q = HR \times SV$. During dehydration exercise, in order for Q to be maintained, HR must increase as SV decreases. If the reduction in SV is minimized through fluid replacement, HR is reduced and the cardiovascular (CV) stress is decreased. This reduction in CV stress enables for a greater potential for maximal exercise intensity when called upon by the active muscles. In order to quantify work done by the cardiovascular system we can calculate training impulse (Avg. HR x Duration). The training impulse for the HCF group was (Mean ± SD) 19167.0 ± 1751.0 total beats, while the HC and Ctrl groups were 19813.0 ± 1599.0 and 19921.0 ± 1381.0 total beats respectively Although not significant ($p>0.05$) the MD (95%CI; ES) for HCF-Ctrl= -754 total beats (-2385, 877; 0.48).

Another interesting point gained from this study was determined when the change in HR for every 1% BML was determined. $\Delta \cdot 1\%BML^{-1}$ for [HCF-HC] and [HCF-Ctrl] was -6.03± 10.31 bpm and -7.41± 8.14 bpm for every 1% respectively. This indicates that there was a slight difference in heart rate between HC and Ctrl when fluid is accounted for. The findings from this study are similar to the findings presented by Adams et al. who examined the $\Delta \cdot 1\%BML^{-1}$ in a meta-analysis examining hyperthermic individuals.

Performance Battery:

Previous studies have shown the ability to maintain increased cardiovascular performance when peripheral cooling is used in hot environments.[72] In the absence of cooling Hargreaves et al.[119] described a state of "circulatory conflict" that arises between skin and active muscle, ultimately leading to a reduction of blood flow to muscle in order to maintain proper thermoregulation. Many studies have investigated this aspect of performance using hot and humid environmental conditions, inhibitory garments and various cooling treatments, however very few have looked at high intensity and short duration activity after peripheral cooling. Grahn et al.[71] showed that by maintaining the optimal temperature for muscle activity by hand cooling, work and strength during pull-up and bench press exercises increase. We found similar improvements in performance through football specific tasks of agility, power, balance and speed.

The sport specific performance variables used in this study are the first to be implemented in a cooling study. So, it was difficult to compare our results to previous literature. However there is a vast knowledge available on ergogenic aids used in sport such as supplements and specified garments.

In the current investigation although no significant differences were observed from PRE to POST for any of the stances BESS Total, SL Firm, SL Foam, Tan Firm, or Tan Foam there was an observed increase in the percentage of errors within group for the Ctrl condition. Ctrl experienced 30.13% more errors following the 90 minute exercise while the HCF group experienced only 7.17% more errors. Although the trial conditions were slightly different, the results from the present study demonstrated that post exercise errors were higher for the HCF group (13.36 ± 5.43), HC group (14.41 ± 5.08) and Ctrl group (18.96 ± 8.46) when compared to the findings of Distefano et al.[115] Distefano et al. found hypohydrated hot (HYH) and euhydrated hot (EUH) individuals while wearing a military pack had BESS scores of 10.83 ± 3.56 and 10.00 ± 2.70 total errors, respectively.[115] Interestingly, although %BML in the current study (HCF=1.60 ± 0.53%) was similar to Distefano et al. (EUH=1.3 ± 0.9%) the errors observed in the Ctrl were much greater even though %BML for HYH was 5.7± 1.6% compared to the Ctrl in our study (3.29 ± 0.68%). It is unlikely that the increased errors are explained by the differences in T_{CORE} given that POST T_{CORE} was similar between the studies. T_{CORE} only differed (MD = Current study minus Distefano et al.) 0.29℃ in the hydrated trials and -0.09℃ in the dehydrated trials. The observed increase in errors may have been a result of the difference in equipment worn in the two trials. The football equipment load distribution is higher with most of the weight being in the helmet and shoulder pads whereas the military pack distributes the load to the hips and lower back resulting in a lower the center of gravity which may possibly improve balance during the BESS compared to the football uniform.

Agility and change-in-direction movements allow players to evade and gain ground when going head-to- head with an opponent.[120] The goal of the offensive player is to cause uncertainty and cause the defensive player to speculate and then evade and outrun them[120] while the defender must react to cues to 1) evade offensive blocks and 2) provide enough force through the opponent to bring them to the ground. One can see that foot speed and reaction time play a large part in the activities football players execute on the field. Using these statements as a basis to compare to, our study showed a slight improvement in foot speed with a 1.48% increase and 3.58% increase from PRE to POST for HCF and HC groups respectively. With an overall improvement for HCF and HC group over the control group of 3.77% and 5.82% respectively we can apply that to a practical scenario where individuals in the former group would have an increased ability to make a change-in-direction movement and then evade their opponent in this scenario (Ctrl group). This brings into account having the blood flow and blood volume to active muscles to provide the oxygen and nutrients needed for anaerobic high intensity movements. Having the energy stores and ability to gain ground on an opponent is only a piece of one's agility performance.

Reaction time takes into account the brains ability to interpret and signal to active body parts providing an increased awareness and control during movement. In conjunction with foot speed improvements, the HCF group had 3.5% faster reaction times from PRE to POST exercise. This translates into a 5.95% improvement over the control group, which leads us to believe that by decreasing the perceived exertion, reducing thermal and thirst sensation it allows individuals to not only move faster, but also react to visual stimulus. This spans from the realm of performance increase to also safety during play, especially toward the end of a practice or game in an uncompensable heat stress situation. As reaction time decreases from a view of fatigue and body positioning it could possibly lead to an increased risk of serious injury.

In the current investigation although no significant differences were observed from PRE to POST during the SPT task, there was an observed percent increase in performance within group for the HCF condition compared to HC and Ctrl conditions. HCF group was able to perform significantly better in POST attempt #1 vs. attempt #2 ($p=0.006$), whereas both HC and Ctrl had mean peak sprint speeds that remained relatively close. This was possibly due to the attenuation of the rise in HR during the exercise session of an avg. of 10 $b \cdot min^{-1}$ lower in the HCF condition compare to HC and Ctrl.

The HCF group ran at peak sprint speeds that were 1.43% faster following the 90 minute exercise. Both HC and Ctrl were slower after exercise with a decrease in speed by 0.68% and 3.56% respectively. With a performance increase of 4.99% (HCF-Ctrl) our study falls in line with others that have found anywhere from 1.1% to 17% improvements in endurance running after receiving body cooling.[121-123] The improvements found after cooling parallels those seen from the ergogenic aids such as caffeine, and correlate to a major advantage in the practical setting of a race or game.[124] Kay et al. found that only small decreases in T_{SKIN} and T_{CORE} are necessary for improved performance.[123] In contrast our study showed significant attenuation in T_{CORE}

(HCF-Ctrl) and although the methodology was different the performance increase was only 1.1% less than the biking performance increase they found.

When applied to football specific performance assessment a 4.99% advantage could equate to the difference in reaching the status of an elite level professional athlete. An approximate 5% advantage while running a 40 yard dash is the difference in a 5.45 and 5.19 second trial for an individual running at 15 mph (7.33 yards·sec^{-1}). Sierer et al. found that the difference between a drafted and undrafted (4.49±0.09 seconds, 5.59±0.11 seconds) skill position player 40-yard dash time was 0.10s, a 21% difference.[125]

Perceptuals:

During the 90 minute treadmill exercise perceived exertion remained similar between HC and Ctrl conditions. Although not significant, it was noted that the HCF condition plateaued at minute 27 and at the end of treadmill exercise minute 87, there was a strong effect size between HCF-HC (ES=0.87). We can speculate that a lower HR from minute 57-87 in the HCF condition causes a decrease in perception of exertion.

With the HCF group receiving fluids in an attempt to minimize %BML during the testing session we expected to see a significant difference ($p \leq 0.005$) in perception of thirst. This was confirmed starting at minute 27 and continuing on until the POST for HCF-Ctrl and minute 42 to POST for HCF-HC. During the course of the 90 minute exercise session the HCF groups thirst perception remained constant and actually decreased at points, while both HC and Ctrl increased steadily during exercise. The fact that participants perceived less thirst in the HCF vs. HC and HCF vs. Ctrl trials and that HC vs Ctrl were closely related to each other corresponds to the USG measurements confirming that we achieved separate hydration statuses between groups.

Although our study found that T_{CORE} remained lower from minute 66 to POST exercise for the HCF condition, thermal sensation, with the exception of minute 87 (significantly lower HCF vs. HC; p=0.08) showed little variation over time between groups. This finding was unexpected, however work from Candas et al. concluded that perception of heat stress is independent of $T_{CORE,}$ and thermal discomfort is more closely tied to sweating rate and skin wettedness.[126] Our study with individuals exercising under uncompensable heat stress, further emphasizes this theory. Although T_{CORE} differed between groups, they all experienced heavy sweating and protective equipment saturated in sweat which is what we observed. Based on thermal sensation there were very minimal differences between groups throughout the 90 minutes of exercise (Figure 39).

In this investigation we found that although not significant ($p>0.05$) perception of fatigue by the participants in the HCF condition was less than both HC and Ctrl with moderate to large ES. HCF experienced reduced fatigue as indicated by the strong ES (HCF-HC=0.82) and moderate ES (HCF- Ctrl=0.52) at the POST time point, the theory behind this is the mitigation of CV drift in the HCF group during exercise.

The goal of this study design was to complete a treadmill exercise protocol as a tool to increase T_{CORE}, and execute the performance battery utilizing full recovery periods for optimal performance (3:1 rest to work). Muscle and CV perception of pain was not intense to our participants because of the lack of intense stress to the CV system and minimal muscle breakdown. Mean difference for perception of pain for the HCF-Ctrl at minute 87 and at the POST time point time point showed strong ES of 0.80 and 0.89. The HCF group's perception of pain plateaued while both HC and Ctrl groups increased. This is likely due to whole body muscle activation and the volume of anaerobic stress experienced in the short duration of POST performance battery.

HCF vs HC and HCF vs. Ctrl showed very large ES for the severity of environmental symptoms. This mimicked the perception of thirst difference between groups in conjunction with T_{CORE} and HR. When we see that ESQ and the perception of thirst are closely related, it stands to reason that the premises for these two variables are the same.

Ratings of Perceived Exertion Scale- No significant differences ($p > 0.005$) were found with rate of perceived exertion (RPE) scores between groups (Figure 37). However there were moderate to strong effect sizes at minute 87 for HCF-HC and HCF-Ctrl with mean differences of [MD (95%CI), ES] -2.50 (-5.49, 0.49), 0.87 and -2.25 (-5.24, 0.74), 0.73 respectively (Table 11).

Environmental Symptoms Questionnaire Scale- HCF was significantly lower than HC and Ctrl ($p=0.001$ and $p=0.002$ respectively) during POST measurement of the environmental symptoms questionnaire (Table 16).

Thirst Scale- There were significantly lower thirst sensation ($p \leq 0.05$) in the HCF group compared to HC and Ctrl groups starting at min 42 (Table 12). There were no significant differences ($p>0.05$) in thirst perception for HC-Ctrl (Figure 38).

Thermal Scale- It was only at min 87 where HCF had a significantly lower ($p=0.03$) thermal perception than HC (Table 13 and Figure 39).

Fatigue Scale- There were no significant differences ($p>0.05$) between groups in perception of fatigue during exercise (Figure 40). A moderate ES (0.52) and strong ES (0.82) were found during min 87 and POST respectively for HCF-HC and a moderate ES (0.52) was seen during POST for HCF-Ctrl (Table 14).

Pain Scale- There were no significant differences ($p>0.05$) between groups in perception of pain during exercise (Figure 41). The ES for HCF-HC were moderate 0.50, 0.60, 0.52, and 0.61 during time points 57, 72, 87 and POST respectively. The ES for HCF-Ctrl were moderate 0.56, 0.70, and 0.58 during time points 42, 57, 72 respectively. Strong ES were found during time points 87 and POST with values of 0.80 and 0.89 respectively (Table 15).

Methodological Considerations:

A limitation of the methodology for treatment groups is that we did not include a hydration only condition. Although this would have given us a clear distinction between the effects of hydration and the effects of peripheral hand cooling, we feel that this does not negate the findings of this study. It is understood that hydration does improve performance in the way of power, strength, and both anaerobic and aerobic activity, so in conjunction with our findings it can be speculated that hand cooling is an additive effect. This synergistic effect can be of practical application to sports team since most already understand the importance of a hydration protocol.

An additional possible concern may be the power of our subject population. We were powered to see a change in core body temperature related to the three conditions of hand cooling only, hand cooling with fluid and control and not for the performance battery. Although data points were trending toward significance over time, the power of our sample affected our ability to see significant differences due to large standard deviations. This is why we looked at percent change from PRE to POST for the performance battery, and as far as practical application it allowed us to make important comparisons and add to the body of knowledge for peripheral hand cooling's effect on performance. Large standard deviations were also seen for our HR data and this could be due to different relative intensities of the treadmill protocol between subjects. The methodology of this study was to keep intensity of exercise the same within subject for each of their 3 testing trials allowing them to reach T_{CORE} of at least 39.0℃. Since everyone's tolerance to the heat is different the intensities of the treadmill protocol had to be different for each subject. So in order to adjust for this we used change in HR to make our comparisons.

PRACTICAL APPLICATIONS:

The purpose of this study was to evaluate the effectiveness of CoreControl™ on physiologic and performance measurements of individuals in the heat with football equipment. The subjects in this study were subjected to severe environmental conditions (34.94±0.31℃ and 38.44±0.11 %RH; full football gear) often seen during football practices or games. The results of our study continue to provide insight into application, effectiveness and scope of the CoreControl™ hand cooling device. It is already understood that proper hydration leads to improved performance, now we can see that in conjunction with hydration, hand cooling decreases body temperature, decreases HR and decreases thermal sensation during exercise. A peripheral hand cooling modality with negative pressure also leads to an improved performance in power, agility and speed tasks after intense exercise in the heat. Our results suggest that this is an adequate cooling modality when paired with proper hydration protocols in mildly hyperthermic football athletes.

CONCLUSION:

Uncompensable heat stress presents a unique environmental obstacle where a peripheral cooling device could potentially improve the outcome. We found that using a peripheral hand cooling modality in uncompensable heat stress lowered physiological variables and improved performance variables. This was shown as a decrease in T_{CORE}, HR, thirst and thermal perception and large MD and effect sizes for BESS, REACT, FF and SPT tasks.

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Tables

Table 4.1 Subject demographic information collected during the familiarization session. Body fat % and lean body mass % measured via DEXA

Demographics of Subjects (mean ± SD) n=12				
Height (cm)	Mass (kg)	Age (yr)	Body fat mass %	Body Lean mass %
178.59 ± 4.69	83.08 ± 9.92	24.25 ± 3.49	18.61 ± 6.71	77.88 ± 6.37

Symbol (**) indicates significantly different HCF versus Ctrl (** $p \le 0.05$).

Table 4.2: Mean difference in Rectal temperature (℃) for all time points during the testing sessions by condition HCF, HC, Ctrl. Variable measured at baseline (PRE); halfway through, at the end of each 12 minute treadmill bout and immediately after treatment session (minutes), and after post-performance tasks (POST). Significant difference HCF-Ctrl starting at minute 66 to POST. Symbol (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

Rectal Temperature Mean Difference (Degrees Celsius) (95%CI) & Effect Size

Condition	Pre	ES	6 min	ES	12 min	ES	15 min	ES	21 min	ES
HCF-HC	0.05 (-0.26, 0.37)	0.16	-0.07 (-0.40, 0.27)	0.20*	-0.18 (-0.50, 0.14)	0.54†	-0.15 (-0.45, 0.15)	0.46*	-0.14 (-0.47, 0.19)	0.41*
HC-Ctrl	-0.02 (-0.33, 0.29)	0.05	-0.04 (-0.37, 0.30)	0.12	0.02 (-0.30, 0.34)	0.06	-0.06 (-0.36, 0.24)	0.20*	-0.12 (-0.44, 0.21)	0.29*
HCF-Ctrl	0.03 (-0.28, 0.34)	0.12	-0.11 (-0.44, 0.23)	0.32*	-0.16 (-0.48, 0.16)	0.53†	-0.21 (-0.51, 0.09)	0.78†	-0.26 (-0.59, 0.07)	0.85‡

Condition	27 min	ES	30 min	ES	36 min	ES	42 min	ES	45 min	ES
HCF-HC	-0.2 (-0.53, 0.13)	0.58†	-0.15 (-0.48, 0.19)	0.44*	-0.21 (-0.55, 0.14)	0.63†	-0.23 (-0.58, 0.12)	0.7†	-0.23 (-0.58, 0.12)	0.70†
HC-Ctrl	-0.03 (-0.36, 0.29)	0.11	-0.07 (-0.41, 0.26)	0.22*	-0.09 (-0.44, 0.26)	0.26*	-0.07 (-0.42, 0.28)	0.20*	-0.05 (-0.40, 0.30)	0.15
HCF-Ctrl	-0.23 (-0.56, 0.09)	0.80‡	-0.22 (-0.55, 0.12)	0.66†	-0.30 (-0.64, 0.05)	0.89‡	-0.30 (-0.66, 0.05)	0.9‡	-0.28 (-0.63, 0.07)	0.82‡

Condition	51 min	ES	57 min	ES	60 min	ES	66 min	ES	72 min	ES
HCF-HC	-0.25 (-0.61, 0.11)	0.80‡	-0.28 (-0.64, 0.08)	0.89‡	-0.23 (-0.59, 0.12)	0.71†	-0.33 (-0.71, 0.05)	1.00‡	-0.37 (-0.76, 0.02)	1.07‡
HC-Ctrl	-0.08 (-0.44, 0.28)	0.21*	-0.04 (-0.40, 0.32)	0.11	-0.11 (-0.47, 0.24)	0.32*	-0.09 (-0.47, 0.29)	0.24*	-0.07 (-0.46, 0.32)	0.19
HCF-Ctrl	-0.33 (-0.69, 0.03)	0.91‡	-0.32 (-0.68, 0.04)	0.88‡	-0.35 (-0.71, 0.01)	0.97‡	-0.42 (-0.80, -0.04)**	1.08‡	-0.44 (-0.83, -0.05)**	1.13‡

Condition	75 min	ES	81 min	ES	87 min	ES	90 min	ES	Post	ES
HCF-HC	-0.31 (-0.68, 0.06)	0.95‡	-0.31 (-0.72, 0.10)	0.84‡	-0.34 (-0.76, 0.07)	0.89‡	-0.3 (-0.73, 0.12)	0.81‡	-0.22 (-0.66, 0.22)	0.52†
HC-Ctrl	-0.11 (-0.48, 0.26)	0.30*	-0.11 (-0.52, 0.30)	0.28*	-0.10 (-0.52, 0.32)	0.24*	-0.17 (-0.60, 0.25)	0.41*	-0.38 (-0.82, 0.06)	0.85‡
HCF-Ctrl	-0.42 (-0.79, -0.05)**	1.13‡	-0.42 (-0.83, -0.01)**	0.99‡	-0.45 (-0.86, -0.03)**	1.04‡	-0.48 (-0.90, -0.05)**	1.08‡	-0.60 (-1.04, -0.16)**	1.43‡

Table 4.3: Calculated heart rate (HR) mean differences with 95% CI and ES for all conditions Hand cooling with fluid (HCF) vs. hand cooling (HC), hand cooling (HC) vs. control (Ctrl), hand cooling with fluid (HCF) vs. control (Ctrl). Symbol (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2). Symbol (**) indicates significantly different HCF versus Ctrl (** p≤0.05).

Heart Rate Mean Difference (95%CI) & Effect Size

Conditions	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	1.25 (-14.46, 16.96)	0.08	-5.17 (-19.69, 9.36)	0.35*	-5.58 (-20.09, 8.93)	0.37*	-6.25 (-19.40, 6.90)	0.47*
HC-Ctrl	-1.25 (-16.96, 14.46)	0.08	0.5 (-14,02, 15.02)	0.04	-0.42 (-14.93, 14.09)	0.03	-0.08 (-13.23, 12.06)	0.01
HCF-Ctrl	0.00 (-15.71, 15.71)	0.00	-4.66 (-19.19, 9.85)	0.33*	-6.00 (-20.51, 8.51)	0.40*	-6.33 (-19.47, 6.81)	0.49*

Conditions	57min	ES	72 min	ES	87 min	ES	Post	ES
HCF-HC	-7.83 (-22.04, 6.38)	0.52†	-9.33 (-23.68, 5.02)	0.62†	-6.08 (-21.75, 9.58)	0.37*	2.58 (-16.76, 21.93)	0.14
HC-Ctrl	-0.41 (-14.63, 13.79)	0.03	-3.33 (-17.68, 11.02)	0.27*	-4.17 (-19.83, 11.50)	0.28*	1.58 (-17.76, 20.93)	0.08
HCF-Ctrl	-8.25 (-22.46, 5.96)	0.58†	-12.67 (-27.02, 1.68)	0.88‡	-10.25 (-25.92, 5.42)	0.72†	4.17 (-23.51, 15.18)	0.26*

Table 4.4: Raw scores (mean ± SD) of all performance tasks for each condition hand cooling with fluid (HCF), hand cooling only (HC), and control (Ctrl). REACT=Reaction Drill, FF= Fast Feet Drill , CMVJ= Counter Movement Vertical Jump, BESS = Balance Error Scoring System.

Performace Task Mean ± SD

	Pre Exercise Measures							
	REACT Time (secs)	**REACT Total Touche**	**REACT Misses**	**FF- Touches**	**BESS**	**SPT**	**CMVJ Ht**	**CMVJ VGRF**
HCF	0.57 ± 0.06	14.17 ± 1.40	0.63 ± 0.80	108.54 ± 6.04	14.42 ± 5.30	15.77 ± 0.71	27.7 ± 5.1	2618.52 ± 956.45
HC	0.55 ± 0.05	14.63 ± 1.11	0.96 ± 0.84	104.54 ± 6.49	13.75 ± 6.06	15.70 ± 0.50	28.0 ± 4.2	2809.95 ± 429.35
Ctrl	0.55 ± 0.04	14.42 ± 0.90	0.54 ± 0.69	108.17 ± 5.73	15.00 ± 6.33	16.07 ± 0.57	28.8 ± 6.4	2864.24 ± 507.88
	Post Exercise Measures							
	REACT Time (secs)	**REACT Total Touche**	**REACT Misses**	**FF- Touches**	**BESS**	**SPT**	**CMVJ Ht**	**CMVJ VGRF**
HCF	0.55 ± 0.06	14.58 ± 1.38	0.96 ± 0.86	110.04 ± 5.97	13.38 ± 5.43	16.00 ± 0.83	30.1 ± 6.1	2952.29 ± 570.49
HC	0.57 ± 0.06	14.21 ± 1.29	0.63 ± 0.71	108.08 ± 6.60	14.41 ± 5.08	15.59 ± 1.29	30.1 ± 6.6	2850.87 ± 564.17
Ctrl	0.56 ± 0.06	14.21 ± 1.29	1.04 ± 0.92	105.75 ± 11.06	18.96 ± 8.47	15.49 ± 0.40	30.7 ± 5.3	2920.92 ± 510.34

Table 4.5: Calculated mean differences for each performance task with 95% CI and ES. Hand cooling with fluid (HCF) vs. hand cooling (HC), hand cooling (HC) vs. control (Ctrl), hand cooling with fluid (HCF) vs. control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2). REACT=Reaction Drill, FF= Fast Feet Drill, CMVJ= Counter Movement Vertical Jump, BESS = Balance Error Scoring System.

Performance Task % Change Mean Difference (95% CI); Effect Size (ES)

	REACT Time %	**ES**	**REACT Total Touches %**	**ES**	**FF- Touches %**	**ES**
HCF-HC	-7.1 (-15.23,1.03)	0.86‡	5.87 (-1.75, 13.48)	0.76†	-2.06 (-8.60, 4.48)	0.40*
HC-CTRL	1.13 (-6.99,9.27)	0.13	-0.01 (-7.63, 7.60)	0.00	5.83 (-0.71, 12.37)	0.82‡
HCF-Ctrl	-5.97 (-14.10, 2.17)	0.88‡	5.85 (-1.76,13.47)	0.82‡	3.77 (-2.77, 10.31)	0.56†

	BESS %	**ES**	**SPT %**	**ES**	**CMVJ Height %**	**ES**	**CMVJ VGRF %**	**ES**
HCF-HC	-20.50 (-52.77, 11.79)	0.84‡	2.11(-2.93,7.17)	0.38*	1.97 (-9.23,13.17)	0.18	3.07 (-3.87, 10.01)	0.41*
HC-CTRL	-16.82 (-49.10, 15.46)	0.47*	2.88 (-2.17,7.92)	0.50†	-1.02 (-12.22,10.18)	0.08	1.99 (-4.94, 8.94)	0.14
HCF-Ctrl	-37.31 (-69.60, -5.03)	1.14‡	4.99 (-0.05,10.04)	1.74‡	0.95 (-10.25,12.15)	0.11	-1.02 (-10.18, 12.22)	0.40*

‡= Strong Effect (0.8)
†= Moderate Efffect (0.5)
*= Small Effect (0.2)

REACT=Reaction Drill
CMVJ= Counter Movement Vertical Jump
BESS = Balance Error Scoring System

Table 4.6: The percent change PRE to POST for performance battery for all conditions HCF, HC, Ctrl. (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2). REACT=Reaction Drill, FF= Fast Feet Drill, CMVJ= Counter Movement Vertical Jump, BESS = Balance Error Scoring System.

% Change Pre to Post (95% CI); Effect Size (ES)

	REACT Time %	ES	REACT Total Touches %	ES	FF- Touches %	ES
HCF-HC	-7.1 (-15.23,1.03)	0.86‡	5.87 (-1.75, 13.48)	0.76†	-2.06 (-8.60, 4.48)	0.40*
HC-CTRL	1.13 (-6.99,9.27)	0.13	-0.01 (-7.63, 7.60)	0.00	5.83 (-0.71, 12.37)	0.82‡
HCF-Ctrl	-5.97 (-14.10, 2.17)	0.88‡	5.85 (-1.76,13.47)	0.82‡	3.77 (-2.77, 10.31)	0.56†

	BESS %	ES	SPT %	ES
HCF-HC	-20.50 (-52.77, 11.79)	0.84‡	2.11(-2.93,7.17)	0.38*
HC-CTRL	-16.82 (-49.10, 15.46)	0.47*	2.88 (-2.17,7.92)	0.50†
HCF-Ctrl	-37.31 (-69.60, -5.03)	1.14‡	4.99 (-0.05,10.04)	1.74‡

Table 4.7: Mean BESS scores by stance (Mean ± SD) for all conditions HCF, HC, and Ctrl.

BESS Score By Stance

Variable	Condition	PRE (Mean SD)	PRE 95%CI	POST (Mean SD)	POST (95%CI)
BESS score: total (errors)	HCF	14.42 ± 5.30	11.05 to 17.78	13.36 ± 5.43	9.93 to 16.83
	HC	13.75 ± 6.05	9.90 to 17.60	14.41 ± 5.08	11.19 to 17.64
	Ctrl	15.00 ± 6.33	10.98 to 19.02	18.96 ± 8.46	13.58 to 24.33
BESS score: SL firm (errors)	HCF	2.04 ± 1.41	1.15 to 2.93	2.46 ± 1.47	1.53 to 3.39
	HC	2.33 ± 1.47	1.40 to 3.27	4.04 ± 2.97	2.16 to 5.93
	Ctrl	3.04 ± 2.10	1.70 to 4.38	4.42 ± 3.38	2.27 to 6.56
BESS score: Tan firm (errors)	HCF	0.46 ± 0.49	0.14 to 0.77	0.58 ± 0.73	0.11 to 1.05
	HC	0.29 ± 0.39	0.04 to 0.54	0.88 ± 0.98	0.25 to 1.49
	Ctrl	0.75 ± 0.89	0.18 to 1.31	0.88 ± 0.86	0.33 to 1.42
BESS score: SL foam (errors)	HCF	8.00 ± 2.49	6.41 to 9.59	7.38 ± 2.94	5.51 to 9.24
	HC	6.63 ± 3.06	4.68 to 8.57	7.67 ± 2.31	6.20 to 9.13
	Ctrl	7.38 ± 3.46	5.18 to 9.57	8.79 ± 2.33	7.31 to 10.27
BESS score: Tan foam (errors)	HCF	3.92 ± 2.49	2.33 to 5.50	3.75 ± 2.62	2.08 to 5.41
	HC	4.50 ± 2.84	2.69 to 6.30	3.67 ± 1.72	2.57 to 4.76
	Ctrl	4.21 ± 2.49	2.62 to 5.79	4.17 ± 2.71	3.25 to 5.08

HCF, hand cooling with fluid; HC, hand cooling; Ctrl, control; SL, single leg; Tan, tandem; Surface, firm and foam

Table 4.8: Calculated mean differences for BESS scores divided into separate parts of the test: single leg (SL) firm, Tandem (Tan) firm surface, single leg (SL) foam surface, Tandem (Tan) foam surface with 95% CI and ES. Hand cooling with fluid (HCF) vs. hand cooling (HC), hand cooling (HC) vs. control (Ctrl), hand cooling with fluid (HCF) vs. control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

BESS Score Mean Difference and Effect Size By Stance

Variable		**PRE** **MD (95%CI)**	**PRE** **ES**	**POST** **(MD; 95%CI)**	**POST** **ES**
BESS score: total (errors)	HCF-HC	0.67 (-5.42 to 6.75)	0.12	-1.04 (-7.73 to 5.66)	0.20*
	HCF-Ctrl	-0.58 (-6.67 to 5.50)	0.20*	-5.58 (-12.28 to 1.11)	0.65†
	HC-Ctrl	-1.25 (-7.3 to 4.84)	0.10	-4.55 (-11.24 to 2.15)	0.79†
BESS score: SL firm (errors)	HCF-HC	-0.29 (-2.03 to 1.45)	0.20*	-1.58 (-4.39 to 1.23)	0.67†
	HCF-Ctrl	-1.00 (-2.74 to 0.74)	0.56†	-1.96 (-4.77 to 0.85)	0.75†
	HC-Ctrl	-0.71 (-2.45 to 1.03)	0.39*	-0.38 (-3.19 to 2.44)	0.12
BESS score: Tan firm (errors)	HCF-HC	0.17 (-0.48 to 0.82)	0.38*	-0.29 (-1.18 to 0.59)	0.35*
	HCF-Ctrl	-0.29 (-0.94 to 0.36)	0.40*	-0.29 (-1.18 to 0.59)	0.38*
	HC-Ctrl	-0.46 (-1.11 to 0.19)	0.66†	0.00 (-0.89 to 0.89)	0
BESS score: SL foam (errors)	HCF-HC	1.37 (-1.75 to 4.50)	0.49*	-0.29 (-2.91 to 2.33)	0.11
	HCF-Ctrl	0.63 (-2.50 to 3.75)	0.21*	-1.42 (-4.02 to 1.20)	0.53†
	HC-Ctrl	-0.75 (-3.87 to 2.37)	0.23*	-1.13 (-3.74 to 1.49)	0.48*
BESS score: Tan foam (errors)	HCF-HC	-0.58 (-3.27 to 2.11)	0.22*	0.08 (-2.70 to 2.87)	0.04
	HCF-Ctrl	-0.29 (-2.98 to 2.40)	0.12	1.33 (-4.11 to 1.45)	0.16
	HC-Ctrl	0.29 (-2.40 to 2.98)	0.11	-1.42 (-4.20 to 1.37)	0.22*

HCF, hand cooling with fluid; HC, hand cooling; Ctrl, control; SL, single leg; Tan, tandem; Surface, firm and foam

Table 4.9: Mean difference for rate of perceived exertion (RPE) during all time points for all groups' hand cooling and fluid (HCF), hand cooling (HC), control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

RPE Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	0.75 (-0.66, 2.16)	0.57†	-1.08 (-3.28, 1.12)	0.5†	-0.33 (-2.84, 2.17)	0.13	-0.92 (-3.5, 1.67)	0.35*
HC-Ctrl	-0.42 (-1.8, 0.99)	0.40*	0.92 (-1.28, 3.12)	0.46*	0.17 (-2.34, 2.67)	0.08	0.58 (-2.00, 3.17)	0.26*
HCF-Ctrl	0.33 (-1.07, 1.74)	0.20*	-0.17 (-2.37, 2.03)	0.07	-0.17 (-2.67, 2.34)	0.06	-0.33 (-2.92, 2.25)	0.13
Condition	**57min**	**ES**	**72 min**	**ES**	**87 min**	**ES**	**Post**	**ES**
HCF-HC	-1.17 (-3.76, 1.43)	0.45*	-1.75 (-4.37,0.87)	0.7†	-2.50 (-5.49, 0.49)	0.87‡	-1.42 (-4.98, 2.15)	0.42*
HC-Ctrl	0.33 (-2.26, 2.93)	0.14	0.17(-2.46, 2.79)	0.07	0.25 (-2.74, 3.24)	0.09	0.08 (-3.48, 3.65)	0.02
HCF-Ctrl	-0.83 (-3.43, 1.76)	0.33*	-1.58 (-4.21, 1.04)	0.6†	-2.25 (-5.24, 0.74)	0.73†	-1.33 (-4.90, 2.23)	0.41*

Table 4.10: Mean difference for thirst during all time points for all groups' hand cooling and fluid (HCF), hand cooling (HC), control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2). (**) indicates significantly different (**p =0.05 for HCF-HC and HCF-Ctrl)

Thirst Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	0.33 (-0.72, 1.38)	0.30*	-1.08 (-2.37, 0.20)	1.02‡	-1.58 (-3.19, 0.03)	0.98‡	-3.08 (-4.66, -1.51)	** 2.52‡
HC-Ctrl	-0.17 (-1.22, 0.88)	0.22*	0 (-1.28,1.29)	0	0.17 (-1.78, 1.44)	1.28‡	0.33 (-1.25, 1.91)	0.19
HCF-Ctrl	0.17 (-0.88, 1.22)	0.14	-1.08 (-2.37, 0.20)	0.74†	-1.75 (-3.36, -0.14)	** 0.24*	-2.75 (-4.33, -1.17)	** 1.80‡
Condition	**57min**	**ES**	**72 min**	**ES**	**87 min**	**ES**	**Post**	**ES**
HCF-HC	-3.58(-5.21, -1.95)	** 2.66‡	-4.25(-5.92,-2.59)	** 3.3‡	-4.83 (-6.63,-3.04)	** 3.03‡	-4.25 (-5.87, -2.63)	** 2.78‡
HC-Ctrl	0.17 (-1.46, 1.80)	0.09	0.25(-1.42, 1.92)	0.13	0.67 (-1.13, 2.46)	0.37*	0.67 (-0.96, 2.29)	0.45*
HCF-Ctrl	-3.42 (-5.05, -1.79)	** 2.23‡	-4.00 (-5.67, -2.34)	** 2.44‡	-4.17 (-5.96, -2.37)	** 2.32‡	-3.58 (-5.21, -1.96)	** 1.8‡

Table 4.11: Mean difference for thermal perception during all time points between hand cooling and fluid (HCF), hand cooling (HC), control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

Thermal Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	0.46 (-0.04, 0.95)	0.86‡	0.13 (-0.34, 0.59)	0.31*	0.17 (-0.48, 0.81)	0.26*	0.04 (-0.62, 0.70)	0.07
HC-Ctrl	-0.42(-0.91, 0.08)	0.93‡	-0.25 (-0.71,0.21)	0.55†	-0.29 (-0.94, 0.36)	0.49*	-0.17 (-0.83, 0.49)	0.23*
HCF-Ctrl	0.04 (-0.45, 0.54)	0.09	-0.13(-0.59, 0.34)	0.26*	-0.13 (-0.77, 0.52)	0.19	-0.13 (-0.79, 0.54)	0.21*

Condition	57min	ES	72 min	ES	87 min	ES	Post	ES
HCF-HC	-0.08(-0.87,0.71)	0.12	-0.33(-1.27,0.60)	0.5†	-0.79 (-1.52,-0.06)	** 1.09‡	-0.63 (-1.57, 0.32)	0.60†
HC-Ctrl	0.04(-0.75,0.83)	0.05	0.33(-0.60, 1.27)	0.32*	0.13 (-0.61, 0.86)	0.19	0.67 (-0.95, 0.95)	0.00
HCF-Ctrl	-0.04 (-0.83, 0.75)	0.05	0.00 (-0.94, 0.94)	0	-0.67 (-1.40, 0.06)	0.91‡	-0.63 (-1.58, 0.32)	0.65†

Table 4.12 Mean difference for fatigue perception during all time points for all groups' hand cooling and fluid (HCF), hand cooling (HC), control (Ctrl). (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

Fatigue Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	0.25 (-0.50, 1.00)	0.30*	0.08 (-1.30, 1.47)	0.06	0.08 (-2.08, 2.25)	0.04	-0.58 (-2.62, 1.46)	0.29*
HC-Ctrl	0.25 (-0.50, 1.00)	0.40*	0.08 (-1.30, 1.47)	0.07	0.08 (-2.08, 2.25)	0.04	0.50 (-1.54, 2.54)	0.24*
HCF-Ctrl	0.50 (-0.25, 1.25)	0.73†	0.17 (-1.22, 1.55)	0.12	0.17 (-2.00, 2.33)	0.07	-0.08 (-2.12, 1.96)	0.04

Condition	57min	ES	72 min	ES	87 min	ES	Post	ES
HCF-HC	-1.00 (-3.49, 1.49)	0.42*	-1.08 (-3.70,1.54)	0.44*	-1.25 (-3.83,1.33)	0.52†	-1.92 (-4.52,0.68)	0.82‡
HC-Ctrl	0.75 (-1.74, 3.24)	0.30*	0.33 (-2.29,2.95)	0.12	0.50 (-2.08, 3.08)	0.19	0.50 (-2.10, 3.10)	0.20*
HCF-Ctrl	-0.25 (-2.74, 2.24)	0.11	-0.75 (-3.37, 1.87)	0.30*	-0.75 (-3.33, 1.83)	0.31*	-1.42 (-4.02, 1.18)	0.52†

Table 4.13: Mean difference in pain perception for HCF-HC, HC-Ctrl and HCF-Ctrl from PRE and POST. (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

Pain Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	12 min	ES	27 min	ES	42 min	ES
HCF-HC	0.04 (-0.28, 0.36)	0.13	-0.29 (-0.96, 0.38)	0.41*	-0.25 (-0.93, 0.43)	0.35*	-0.33 (-1.14, 0.47)	0.47*
HC-Ctrl	0.04 (-0.28, 0.36)	0.14	0.13 (-0.55, 0.80)	0.18	0.13 (-0.56, 0.81)	0.18	-0.08 (-0.89, 0.72)	0.10
HCF-Ctrl	0.08 (-0.24, 0.40)	0.27*	-0.17 (-0.84,0.51)	0.30*	-0.13 (-0.81, 0.56)	0.23*	-0.42 (-1.22, 0.39)	0.56†

Condition	57min	ES	72 min	ES	87 min	ES	Post	ES
HCF-HC	-0.38 (-1.22, 0.47)	0.50†	-0.50 (-1.40,0.40)	0.60†	-0.42 (-1.29,0.45)	0.52†	-0.63 (-1.89,0.64)	0.61†
HC-Ctrl	-0.17 (-1.02, 0.68)	0.18	0.04 (-0.86,0.44)	0.04	-0.21 (-1.08, 0.66)	0.22*	-0.46 (-1.73, 0.81)	0.32*
HCF-Ctrl	-0.54 (-1.39, 0.31)	0.70†	-0.46 (-1.36, 0.94)	0.58†	-0.63 (-1.49, 0.24)	0.80‡	-1.08 (-2.35, 0.19)	0.89‡

Table 4.14: Mean difference in ESQ with HCF-HC, HC-Ctrl and HCF-Ctrl for PRE and POST. (‡) indicates strong effect size (0.8). Symbol (†) indicates moderate effect size (0.5). Symbol (*) indicates small effect size (0.2).

ESQ Mean Difference ± (95%CI) & Effect Size

Condition	Pre	ES	Post	ES
HCF-HC	1.00 (-1.19, 3.19)	0.43*	-11.17 (-18.08,-4.26)	** 1.55‡
HC-Ctrl	-0.17 (-2.36, 2.02)	0.09	0.83 (-6.08, 7.74)	0.11
HCF-Ctrl	0.83 (-1.36, 3.02)	0.37*	-10.33 (-17.24, -3.43)	** 2.04‡

Figures

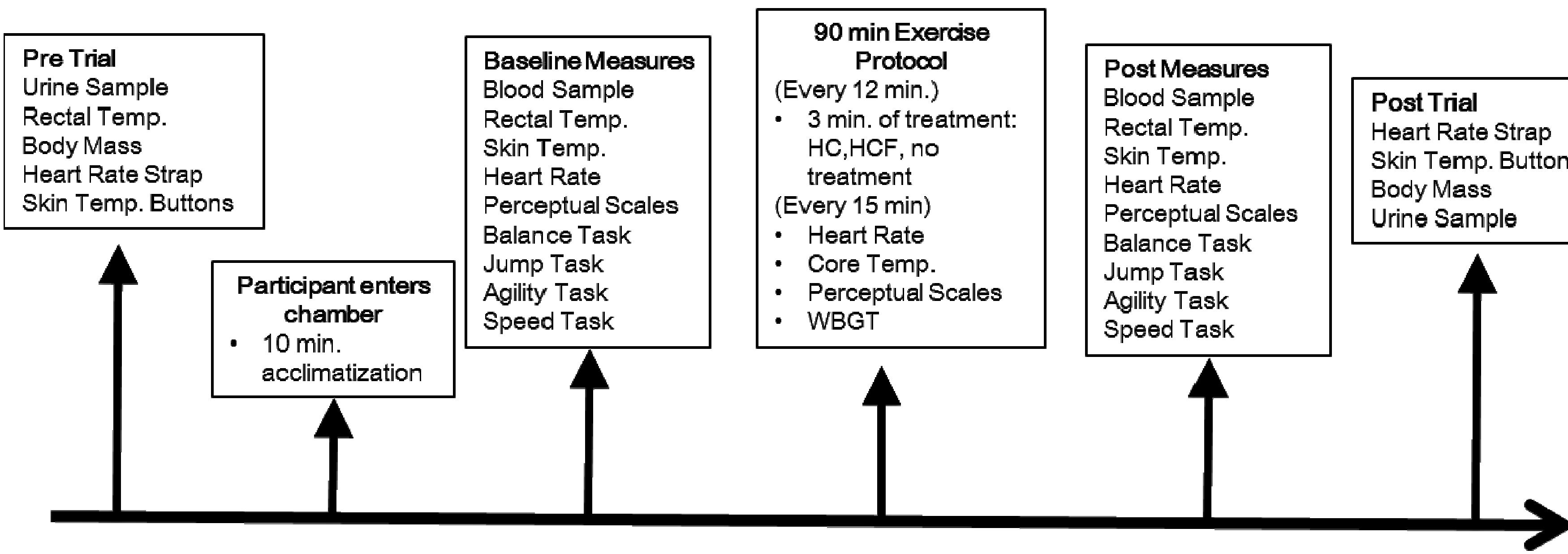


Figure 4.1: Flow chart for all three testing sessions. Only variability during the sessions was the treatment received during each of the 6 exercising bouts for HCF, HC, Ctrl groups.

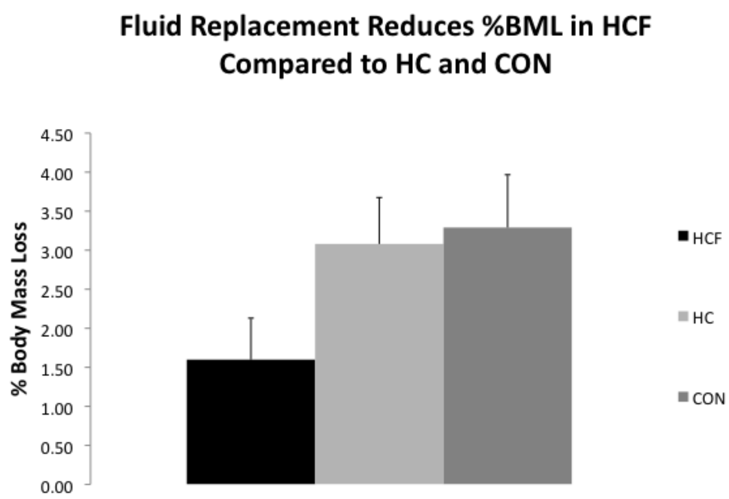


Figure 4.2: Calculated % Body Mass Loss (%BML) by participant for all conditions. The mean %BML difference between groups for HCF vs. HC, HCF vs. Con and HC vs. Con was -1.48,-1.69 and-0.21 respectively. * Significant differences between HCF, HC vs. Control ($p \leq 0.05$)

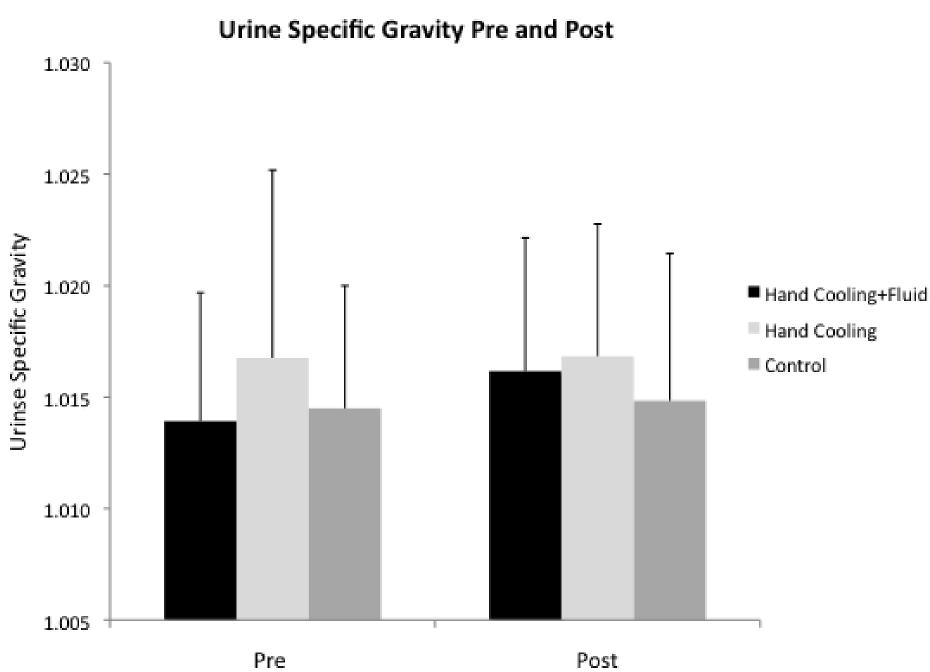
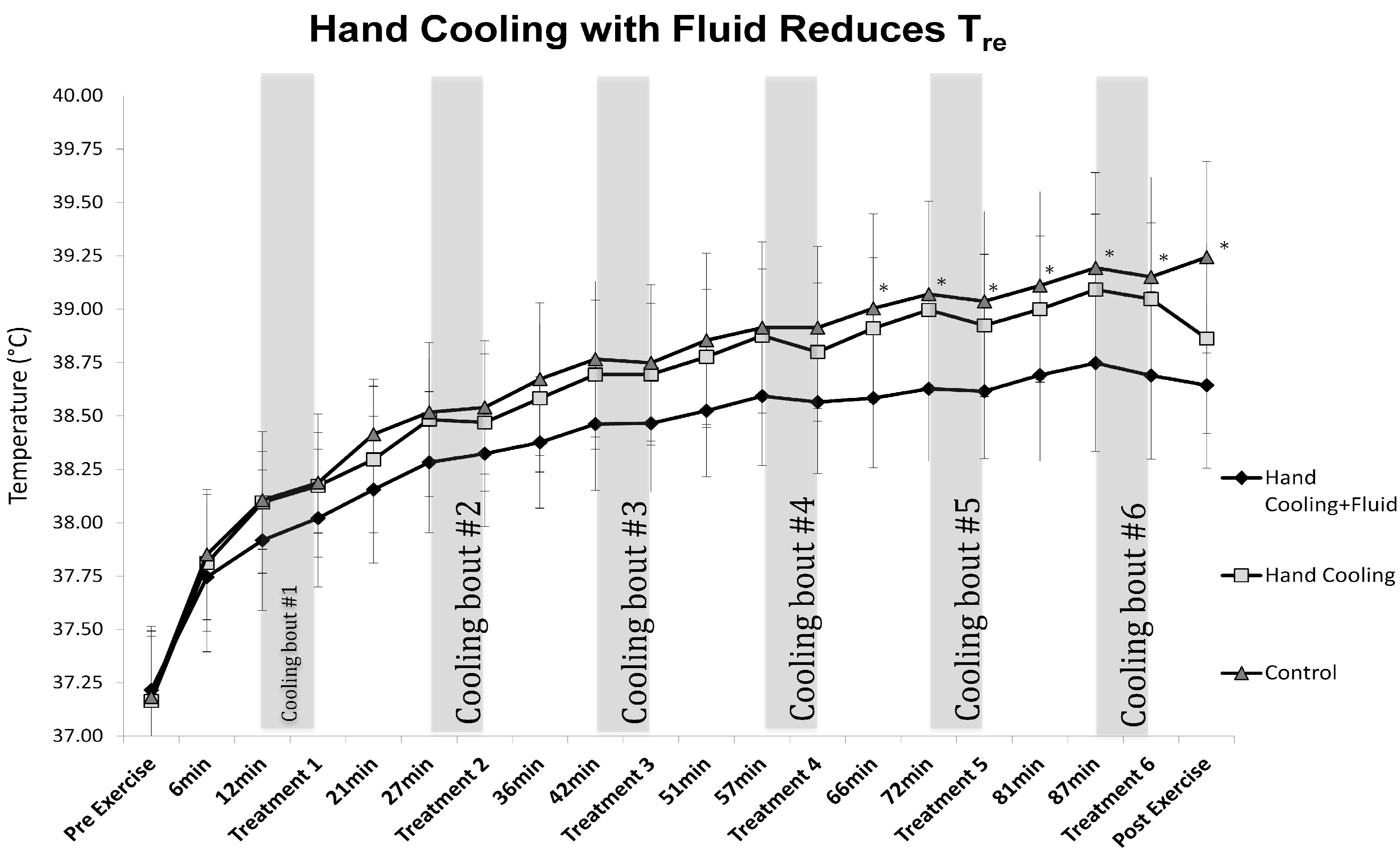
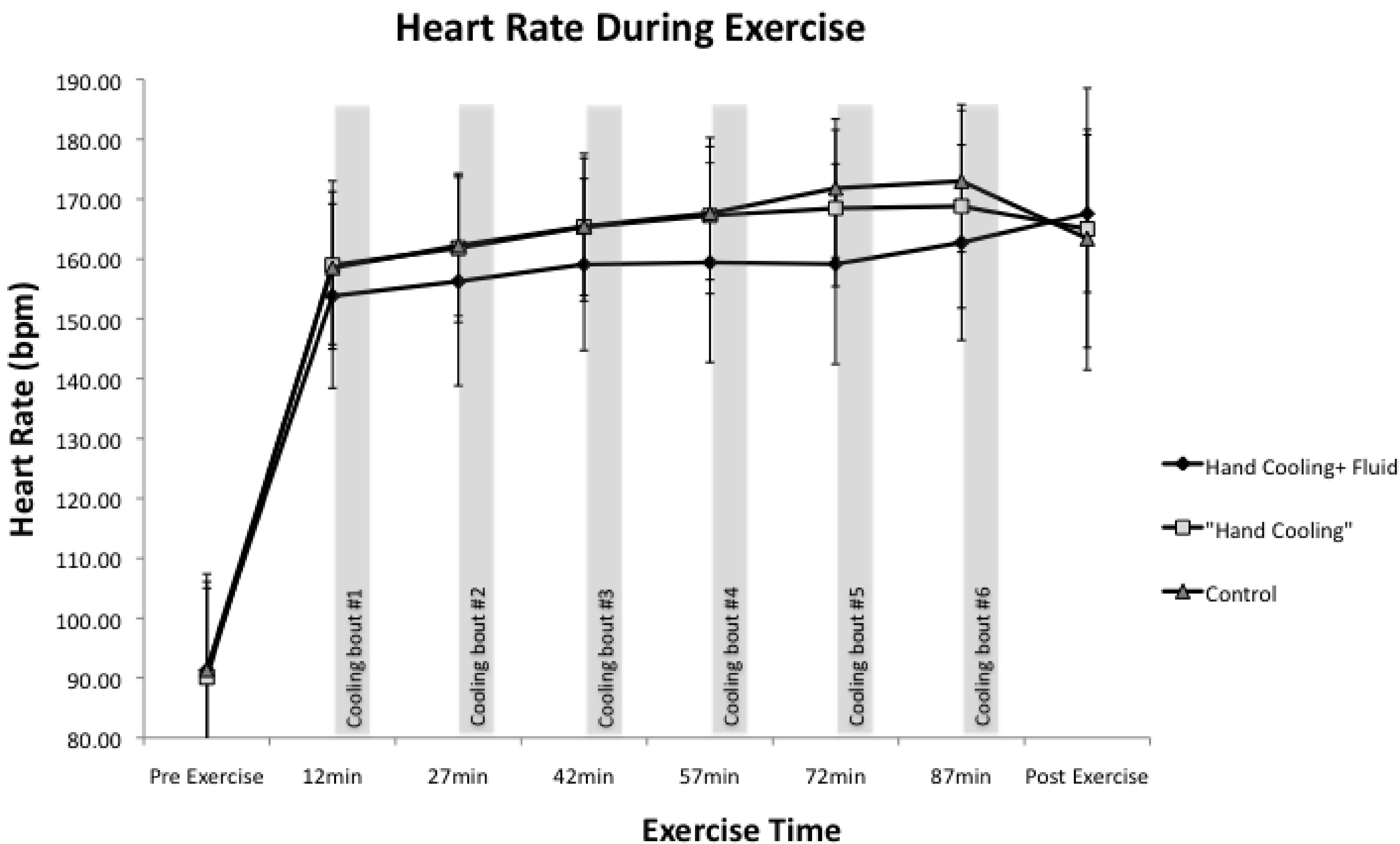


Figure 4.3: Mean urine specific gravity (USG) for all conditions hand cooling with fluid (HCF), hand cooling only (HC), control (CON). No significant difference for mean PRE or POST USG (p=0.00). *= Significant differences between HCF, HC vs. Control ($p \leq 0.05$).



* = indicates significantly different than HCF


Heart Rate During Exercise
Heart Rate (bpm)
190.00
180.00
170.00
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
Cooling bout #1
Cooling bout #2
Cooling bout #3
Cooling bout #4
Cooling bout #5
Cooling bout #6
Pre Exercise
12min
27min
42min
57min
72min
87min
Post Exercise
Exercise Time
Hand Cooling+ Fluid
"Hand Cooling"
Control

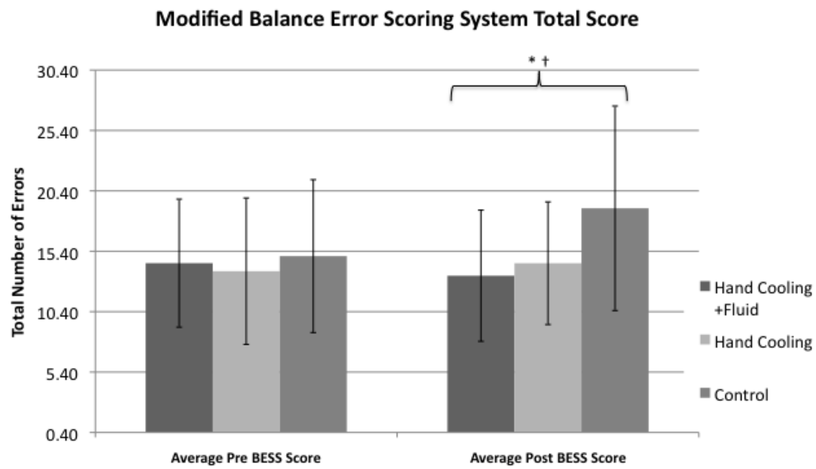


Figure 4.6: Modified BESS Scores for PRE and POST for all conditions HCF, HC, Con. (*)= significant main effect for time (p=0.048). (†) = significant group x time interaction (p=0.033)

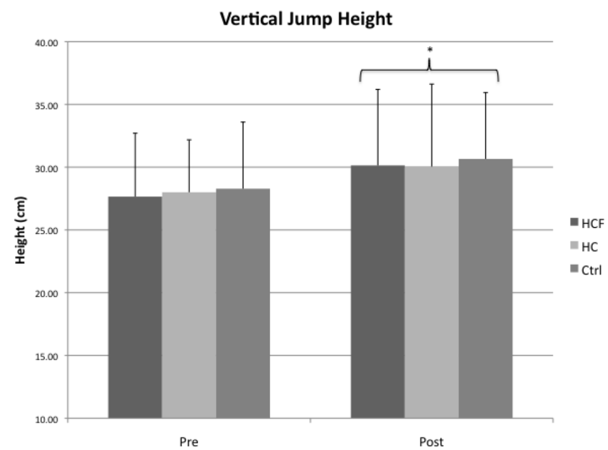


Figure 4.8: Vertical jump height in centimeters for PRE to POST by condition. (*)= significant main effect for time (p=0.008)

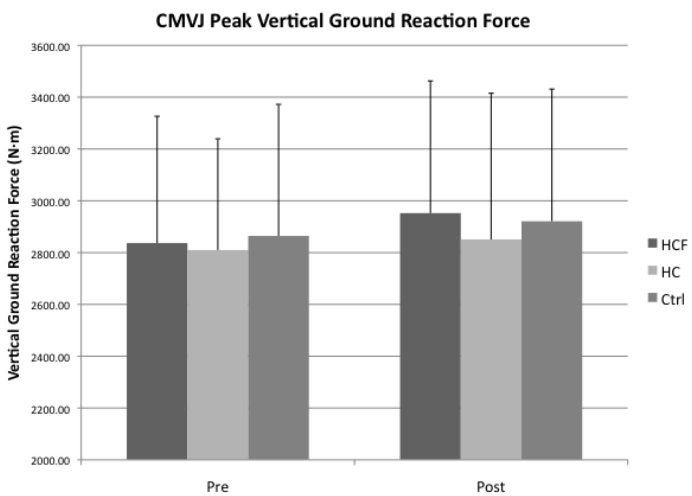


Figure 4.7: CMVJ (Counter Movement Vertical Jump) peak VGRF (Vertical Ground Reaction Force) from PRE to POST. (*) significantly different (p<0.05)

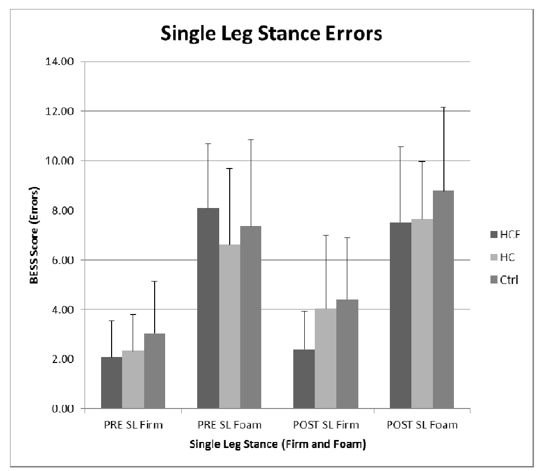


Figure 4.9: Number of errors for PRE and POST single leg stance (SL)

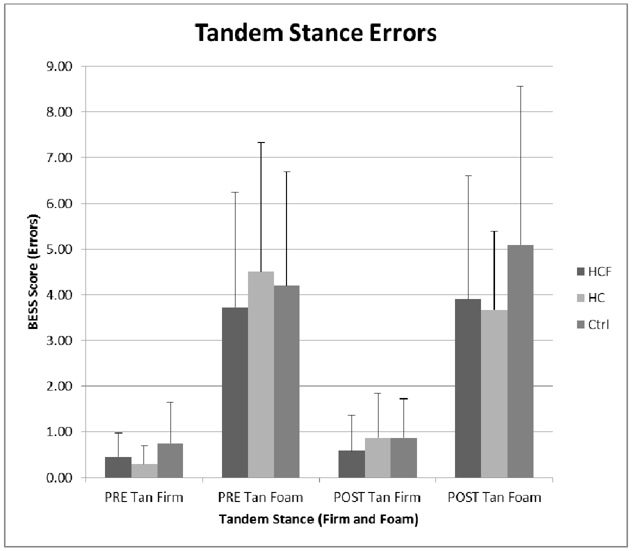
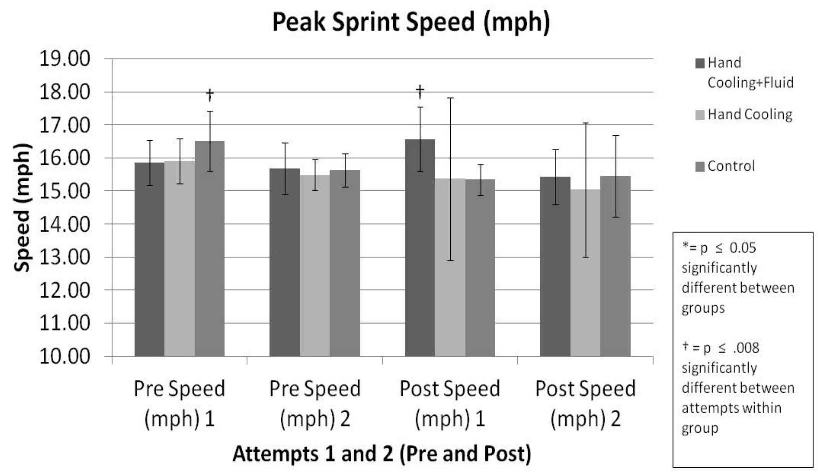


Figure 4.10: Number of errors for PRE and POST tandem stance (TAN) tasks (foam and firm) for all conditions HCF, HC, Ctrl.



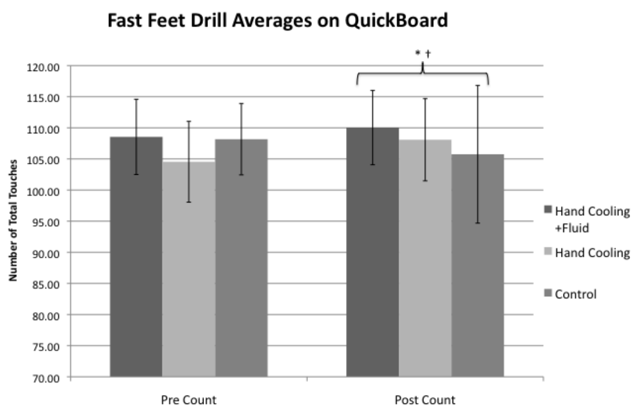


Figure 4.11: Number of touches in 30 seconds during the Quickboard Count Drill for PRE and POST for all conditions HCF, HC, Con. (*)= significant main effect for condition (p=0.022). (†) = significant group x time interaction (p=0.014)

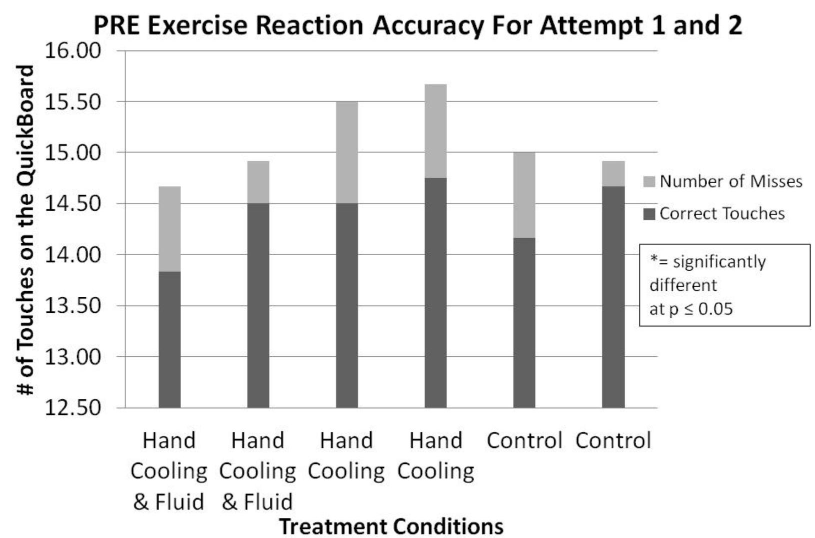


Figure 4.13: Mean pre exercise (PRE) reaction drill (REACT) accuracy measures for all conditions hand cooling and fluid (HCF), hand cooling (HC) and control (Ctrl). The total number of touches on the QuickBoard is divided into number of misses and correct touches. There are no significant differences between groups or within groups between attempts.

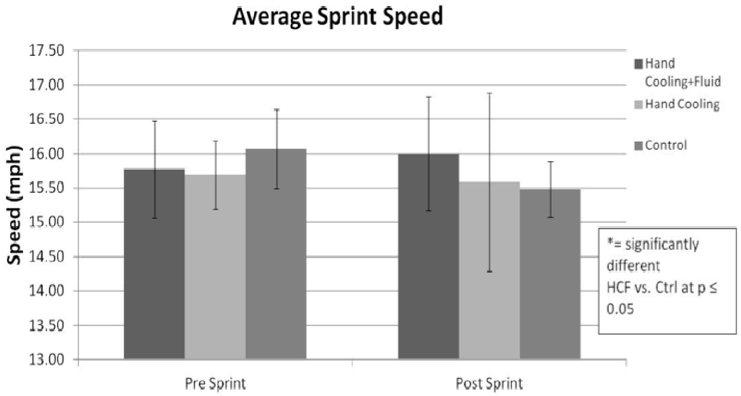


Figure 4.14- Average sprint speed of attempts #1 and #2 for PRE and POST for all conditions HCF, HC, Con. % difference between PRE and POST for HCF, HC, Con are 1.43% faster, 0.68% slower and 3.56% slower respectively.

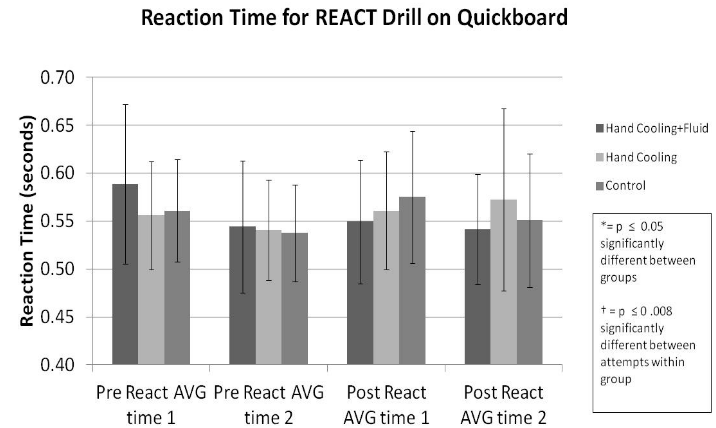


Figure 4.16: Reaction time by condition for attempt 1 and attempt 2 for PRE and POST exercise.

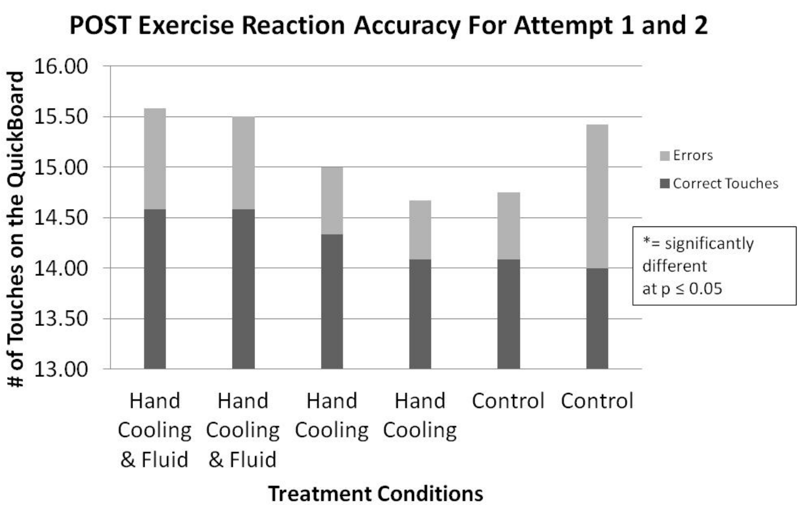


Figure 4.15: POST exercise reaction accuracy for attempt 1 and 2 by group

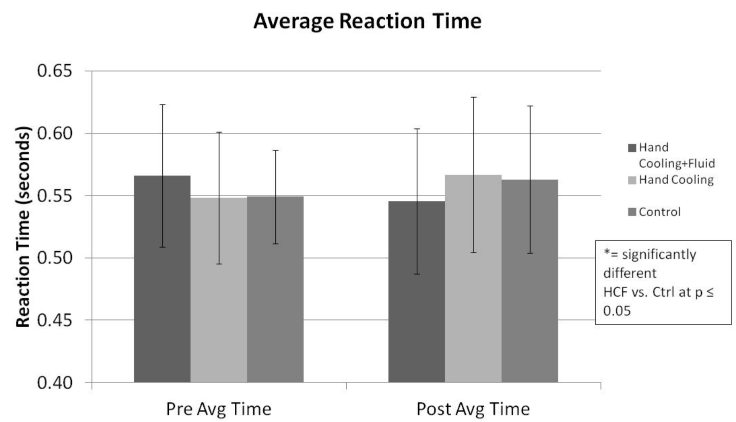


Figure 4.17: Average reaction times for react drill (REACT) during attempts #1 and #2 for PRE and POST for all conditions hand cooling and fluid (HCF), hand cooling (HC) and control(Ctrl).

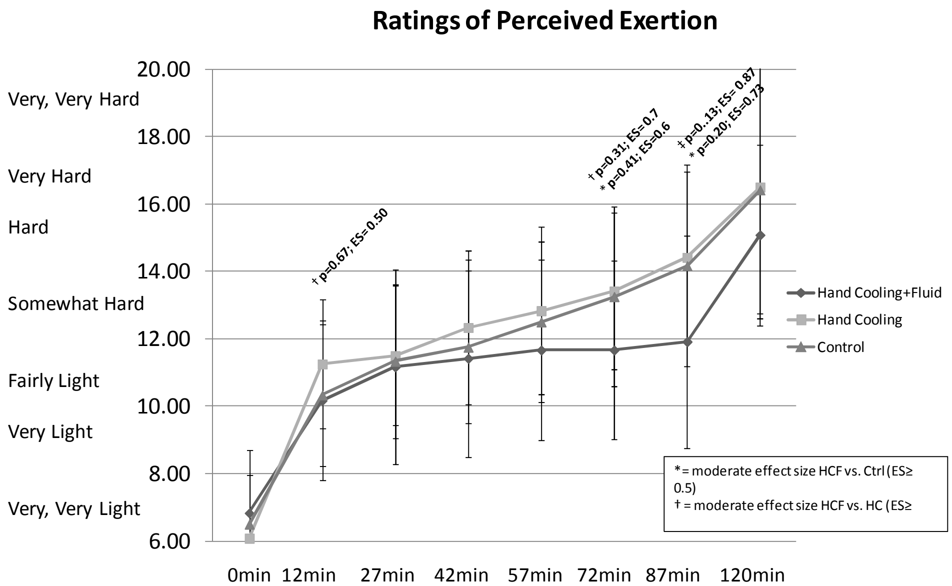


Figure 4.18: No significant differences (p≤ 0.005) between groups HCF, HC, Ctrl.

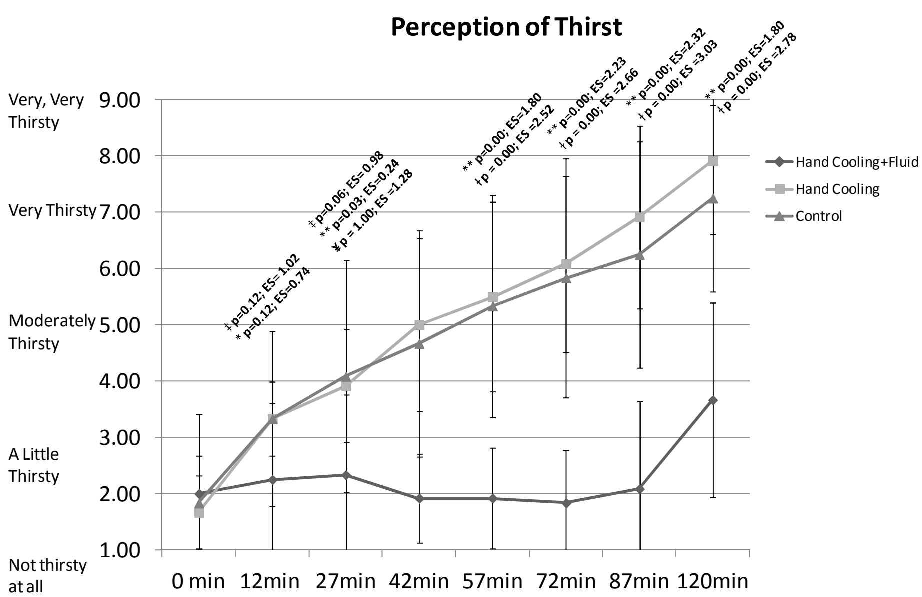


Figure 4.19: (*) = moderate effect size HCF vs. Ctrl (ES≥ 0.5). (†)= moderate effect size HCF vs. HC (ES≥ 0.5). (‡) = strong effect size HCF vs. HC (ES≥ 0.8). (¥) = strong effect size HC vs. Ctrl (ES≥ 0.8). (**) indicates significantly different HCF vs. Ctrl at (p ≤ 0.05). (□) = significantly different HCF vs. HC (p ≤ 0.05).

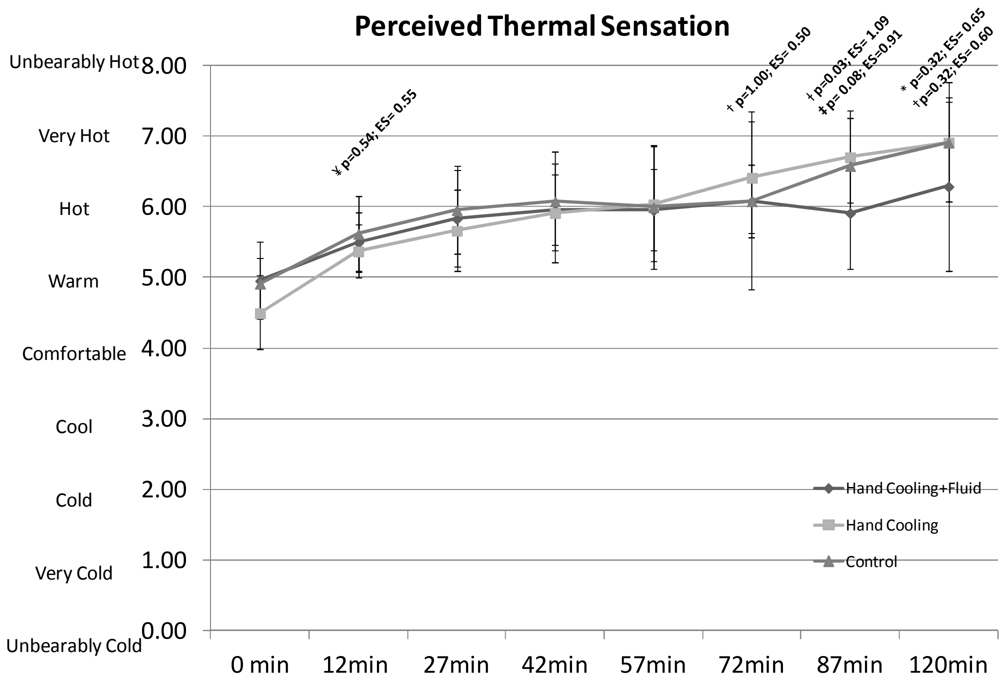


Figure 4.20: Thermal perception during exercise. (*) = moderate effect size HCF vs. Ctrl (ES≥ 0.5). (†)= moderate effect size HCF vs. HC (ES≥ 0.5). (‡)= strong effect size HCF vs. HC (ES≥ 0.8). (¥) = strong effect size HC vs. Ctrl (ES≥ 0.8). (**)= significantly different HCF vs. Ctrl at (p ≤ 0.05). (□)= significantly different HCF vs. HC (p ≤ 0.05).

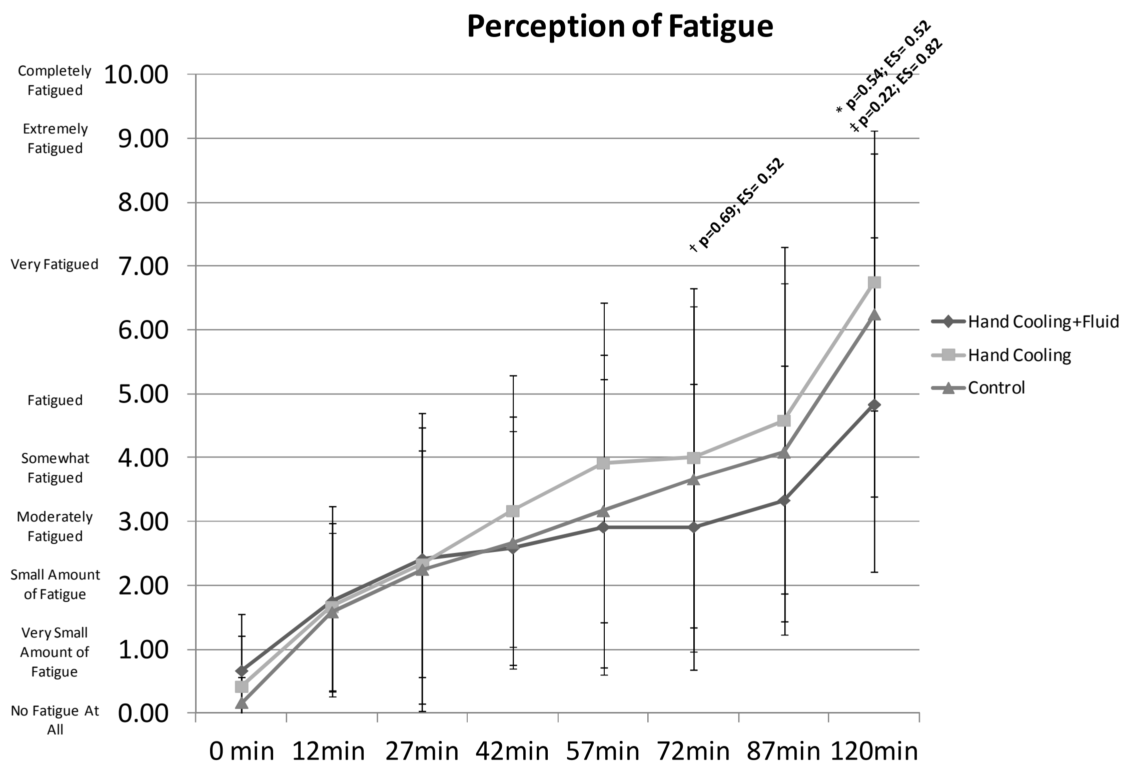


Figure 4.21: Fatigue perception during exercise. (*)= moderate effect size HCF vs. Ctrl (ES ≥ 0.5). (†)= moderate effect size HCF vs. HC (ES≥ 0.5). (‡)= strong effect size HCF vs. Ctrl (ES ≥ 0.8).

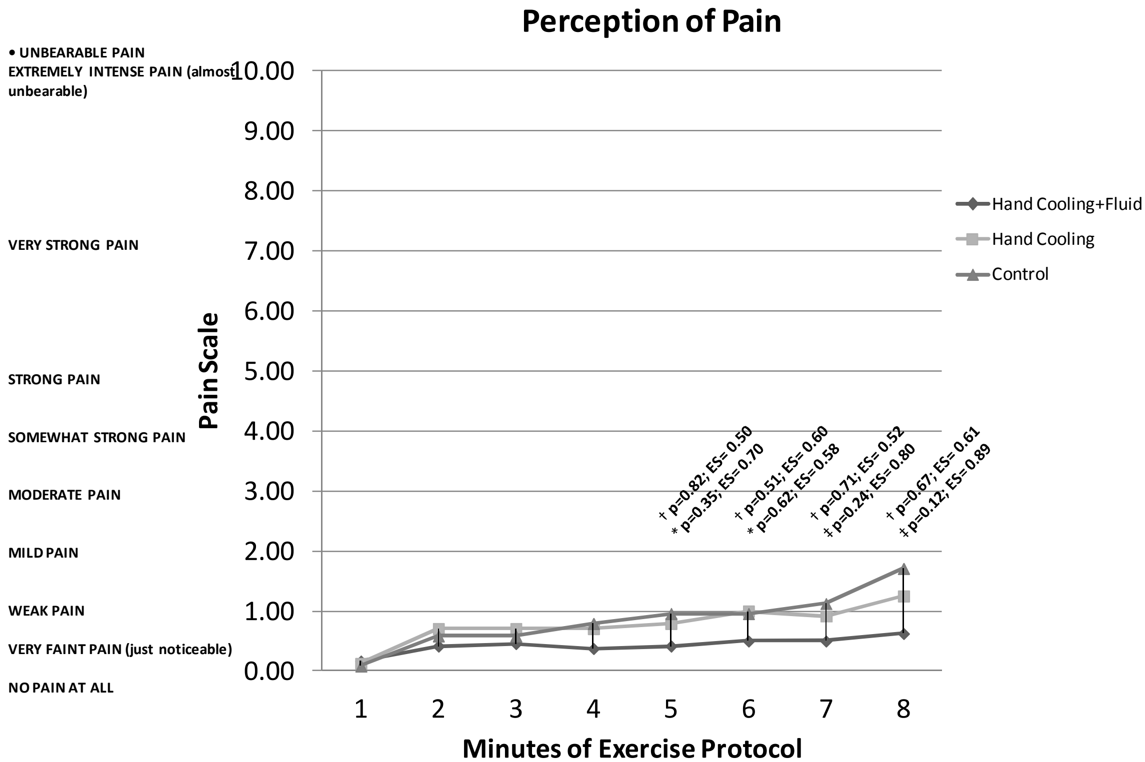


Figure 4.22: (*) = moderate effect size HCF vs. Ctrl (ES≥ 0.5). (†)= moderate effect size HCF vs. HC (ES≥ 0.5). (‡)=strong effect size HCF vs. Ctrl (ES≥ 0.8).

CHAPTER 4: FUTURE DIRECTIONS/ SUMMARY

The purpose of these investigations was multi-factorial. The primary aim was to extensively examine the influence of peripheral hand cooling on body temperature during exercise in football. The secondary aim was to examine the influence of cooling on measures of performance in the laboratory as well as the quantification of field-based GPS measures of performance and the tertiary aim was to closely examine the stress and inflammatory response during uncompensable heat stress. Last, we aimed to examine the reduction in markers of stress and inflammation in response to hand cooling and hand cooling with fluid replacement. In order to increase our understanding of peripheral hand cooling, we developed a research plan to test the device in both field and laboratory settings. In both settings, we devised a research plan that would answer our hypothesis with the main goal to determine the effectiveness of the device in football.

Currently a variety of cooling modalities and technology exits for players, coaches and members of the sports medicine team to enhance thermoregulation during activity. It is important that effective cooling modalities be utilized to not only keep athletes safe, but to enhance performance and recovery. Researchers and coaches are increasingly aware of the need to keep their athletes cool to prevent heat-related illness and welcome the performance and recovery benefits.

The main findings from these present investigations are as follows:

During uncompensable heat stress in the laboratory:

- Peripheral hand cooling with hydration demonstrated improved thermoregulation than the control condition.
- Peripheral hand cooling alone was similar to control, but was not different than hand cooling with fluid which suggests that hand cooling may provide some effect.
- Peripheral hand cooling with hydration resulted in a significant interaction between the three groups over time for IL-8, moderate to large reductions in the inflammatory response for IL-6, and moderately lower circulating cortisol levels.
- Post exercise body temperature was positively associated with the changes observed in %BML, IL-6 and cortisol.
- Peripheral hand cooling with hydration improved performance tasks an average of 5% and 8% compared to hand cooling alone and control respectively. A large percentage of these performance improvements were attributed to balance related changes.

During pre-season football practice in Arkansas:

- Peripheral hand cooling during breaks was not effective in the reduction of T_{GI} or heart rate during exercise.
- Peripheral hand cooling during breaks resulted mixed improvements and detriments for both perceptual and performance measurements from day to day.

- Both locations consumed large amounts of fluid during practice on a daily basis, which may have altered performance and temperature as well.
- Environmental conditions were unseasonably cool compared to meteorological data from previous years, which may have minimized the effectiveness of the device to remove heat from the body.

When both investigations are compared it would appear that the hand cooling device coupled with fluid ingestion was effective in the reduction in body temperature under uncompensable heat stress conditions however in compensable conditions, limited effectiveness was present. Mechanisms for the observed changes in the laboratory may be due to the 1) heat removal from the ingested fluid or 2) the increased blood volume delivered to the cooling device resulting in increased blood volume cooled. Future research should aim to isolate these effects through calculated heat removal from fluid, and add a fourth trial consisting of fluid only during breaks. Furthermore, the cooling duration should be examined to determine if there is a dose-response relationship or an optimal cooling time. Last field research investigations should be conducted in more stressful environmental conditions with increased control of practice components and controlled hydration and nutritional intake.

Appendix A. Medical History Questionnaire For Field and Laboratory Studies

Appendix B

HUMAN PERFORMANCE LABORATORY MEDICAL HISTORY QUESTIONNAIRE

Study: The Effect of Intermittent Hand Cooling On Body Termperature and performance During High School Football practice in the Heat   Subject # ____________

Name ____________ Sex ______ Age ______ DOB ____________

Street ____________

City ____________ State ______ Zip ______ Phone ____________

Email ____________

PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS AND PROVIDE DETAILS FOR ALL "YES" ANSWERS IN THE SPACES AT THE BOTTOM OF THE FORM.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2.	Has your doctor ever denied or restricted your participation in sports or exercise for any reason?
☐	☐	3.	Do you ever feel discomfort, pressure, or pain in your chest when you do physical activity?
☐	☐	4.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	5.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	6.	Does your heart race or skip beats during exercise?
☐	☐	7.	Has a doctor ever ordered a test for you heart? (i.e. EKG, echocardiogram)
☐	☐	8.	Has anyone in your family died for no apparent reason or died from heart problems or sudden death before the age of 50?
☐	☐	9.	Have you ever had to spend the night in a hospital?
☐	☐	10.	Have you ever had surgery?

11. Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated.

☐ High blood pressure	☐ Elevated cholesterol	☐ Diabetes
☐ Asthma	☐ Epilepsy (seizures)	☐ Kidney problems
☐ Bladder Problems	☐ Anemia	☐ Heart problems
☐ Coronary artery disease	☐ Lung problems	☐ Chronic headaches

YES	NO		
☐	☐	12.	Have you ever gotten sick because of exercising in the heat? (i.e. cramps, heat exhaustion, heat stroke)
☐	☐	13.	Have you had any other significant illnesses not listed above?
☐	☐	14.	Do you currently have any illness?
☐	☐	15.	Do you know of <u>any other reason</u> why you should not do physical activity?

16. Please list all medications you are currently taking. Make sure to include over-the-counter medications and birth control pills.

Drugs/Supplements/Vitamins	Dose	Frequency (i.e. daily, 2x/day, etc.)

YES	NO	16. Questions Related to Complications with Gastrointestinal pill
☐	☐	Do you have suspected obstructive disease of the gastrointestinal (GI) tract? (diverticulitis or inflammatory bowel disease?)
☐	☐	Do you have impaired gag reflex?
☐	☐	Have you had previous GI surgery?
☐	☐	Do you believe you might undergo MRI scanning in the next 10 days?
☐	☐	Do you have hypomotility disorder of the GI tract?
☐	☐	Do you have a cardiac pacemaker or other implanted electro medical device?

DETAILS: ____________

17. Please list all allergies you have.

Substance	Reaction

YES	NO	19. Have you smoked?	If yes, #/day	Age Started	If you've quit, what age?
☐	☐	Cigarettes			
☐	☐	Cigars			
☐	☐	Pipes			

20. Do you have a family history of any of the following problems? If yes, note who in the space provided.

- ☐ High blood pressure ________
- ☐ High cholesterol ________
- ☐ Diabetes ________
- ☐ Heart disease ________
- ☐ Kidney disease ________
- ☐ Thyroid disease ________

21. Please check the box next to any of the following body parts you have injured in the past and provide details.

- ☐ Head ________
- ☐ Neck ________
- ☐ Upper back ________
- ☐ Lower back ________
- ☐ Chest ________
- ☐ Hip ________
- ☐ Thigh ________
- ☐ Knee ________
- ☐ Ankle ________
- ☐ Foot ________
- ☐ Calf/shin ________
- ☐ Shoulder ________
- ☐ Upper arm ________
- ☐ Elbow ________
- ☐ Hand/fingers ________

YES	NO	
☐	☐	22. Have you ever had a stress fracture?
☐	☐	23. Have you ever had a disc injury in your back?
☐	☐	24. Has a doctor ever restricted your exercise because of an injury?
☐	☐	25. Do you currently have any injuries that are bothering you?

26. Do you consider your occupation as?

- ☐ Sedentary (no exercise)
- ☐ Inactive-occasional light activity (walking)
- ☐ Active-regular light activity and/or occasional vigorous activity (heavy lifting, running, etc.)
- ☐ Heavy Work-regular vigorous activity

27. List your regular physical activities

Activity	How often do you do it?	How long do you do it?	How long ago did you start?

ADDITIONAL DETAILS:

Appendix B.

University of Connecticut and The Korey Stringer Institute
Research Photo Release Form

I, _______________ hereby authorize the University of Connecticut and the Korey Stringer Institute and those acting pursuant to its authority ("UCONN/KSI") to record my likeness and/or voice on any medium ("recordings") including but not limited to video, audio, photographic, digital, and electronic mediums during my participation in this research study.

I also understand that UCONN/KSI's use of these recordings can include but is not limited to reproducing, exhibiting, performing, displaying, altering or distributing the recordings ("use"). I hereby authorize UCONN/KSI to use these recordings in any medium (e.g. print publications, videotapes, CD-ROM, Internet), in any manner, and for any purpose that supports the mission of KSI including educational, research, promotional, and advertising efforts.

I understand that all such recordings, in whatever medium, shall remain the property of UCONN/KSI. I waive any rights, claims or interests I may have to control the use of my identity or likeness in these recordings, and agree that any use may be made without compensation or additional consideration to me. I release UCONN/KSI from liability for any violation of any personal or proprietary right I may have in connection with its uses. My name will not be used in any publication. I will make no monetary or other claim against UCONN/KSI for the use of the photograph(s)/video.

I represent that I am competent to execute this Agreement and I have read and understood this entire document before signing below, fully intending to be legally bound by its terms **(Youth under 18 years of age must have a parent/legal guardian signature.)**.

Name: ____

Address: ____

Street

____

City State Zip

Phone: ____

Signature of Minor : ______________________ Date: ____________

Parent Signature: ______________________ Date: ____________

Appendix C:

Exercise and Football History

Subject #:___________________

Exercise History
How many hours a week of activity outside did you do before football started? _____hours/week

Did you have a job that required you to be outside during the day?   YES   NO

What type(s) of activity did you do outside most often this summer?(circle)

Running/Jogging   Swimming   Manual Labor   Bicycling/Skateboarding   Team sports (basketball, football, etc.)

Football History
At what age did you start playing football? _____years old

How many seasons have you played football? (not including this year) ______season(s)

This season
How many practices have you participated in so far? _______ practices

Did you participate in any team conditioning sessions during the summer? _______ sessions

How long have your practices lasted? ____________ hours

What position(s) do you play or intend to play this year? ______________________________

____

____

Appendix D.

Parental Permission Form for Participation in a Research Study

Principal Investigator(s): Douglas J. Casa, PhD, ATC and Brendon P. McDermott, PhD, ATC
Student Researcher: Robert Huggins, M.Ed, ATC
Study Title: The Effect of Intermittent Hand Cooling on Body Temperature and Performance During High School Football Practice in the Heat

Introduction

Reducing body temperature via cooling during exercise in the heat has been shown to improve performance in measures of strength and endurance. However, the use of an easy to use hand-cooling device on keeping body temperature from getting to high and performance during high school football practices in the heat has not been shown.

Your child is invited to participate in a research study to examine the influence of hand cooling on body temperature and performance during high school football practices. We will be comparing differences in core body temperature and heart rate as well as performance measures such as distance covered, average speed, and max speed in two different groups during practice sessions. We also plan to collect information on your child's levels of fatigue, sleep, pain, muscle soreness, thirst, thermal sensation, and recovery before and after training sessions. Last, we will collect measures of hydration to determine your child's hydration status over the course of the practice sessions. Information will be collected over the course of 4 successive days of pre-season practices.

Your child is being asked to participate because he/she is a healthy athlete who is participating in pre-season football practices at their high school. Also you are interested in obtaining your child's measures of hydration and measures of performance such as speed zones, distance traveled, and accelerations during practices.

This permission form will give you the information you will need to understand why this study is being done and why your child is being invited to participate. It will also describe what your child will be asked to do to participate and any known risks, inconveniences or discomforts that your child may have while participating. We encourage you to take some time to think this over and to discuss it with your child, other family members, friends and, if applicable, your child's doctor. We also encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign this form, your child will be asked to sign the form and it will be a record of your permission to allow your child to participate. You will be given a copy of this form.

Why is this study being done?

The purpose of this study is to look at how hand cooling changes body temperature and feelings during pre-season football practice. Approximately 30 players on the team will be asked to participate. Half of the players on each team will get hand cooling during their regularly scheduled breaks during practice while the other half of the players will go about their normal practice. We will also look at markers of hydration and muscle damage in the urine. Furthermore performance such as distance covered, speed zones, and accelerations will be recorded by one of two different GPS units.

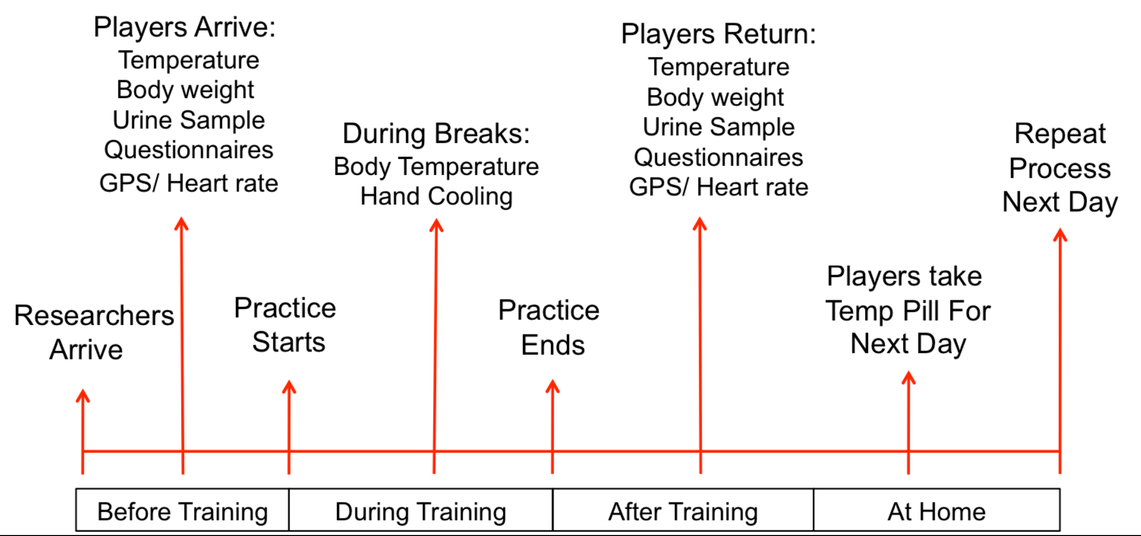
Research questions include:

1. What is the effect of hand cooling during exercise breaks on reducing the rise of body temperature during pre-season football practice?
2. What is the effect of hand cooling during exercise breaks on measures of performance such as total distance covered, efforts achieved, speed zones obtained, and player load?
3. What is the effect of hand cooling during exercise on heart rate?
4. What is the effect of hand cooling during exercise on markers of stress and muscle breakdown found in the urine?
5. What is the effect of hand cooling during exercise breaks on perceptual measures such as thirst, thermal sensation, fatigue, recovery, pain, and rating of perceived exertion?

What are the study procedures? What will my child be asked to do?

If you give permission for your child to take part in this study, he/she will be asked to partake in 4 days of data collection that will take place before and after each practice session. During each day multiple practices may take place depending on the individual team schedule for those 4 days. There is the potential for 6 practice sessions based on the heat acclimatization guidelines set forth by the state of Arkansas that mandates no more than 2 practices per day and when 2 practices per day take place, they must be followed by a single practice the following day. Your child will be randomly assigned to one of the following groups. 1.) no hand cooling during breaks 2.) hand cooling during breaks. Your child will go about your normal practice and scheduled breaks built in by your coaches. There will be no interference with practice. If your child is in the hand-cooling group you will be asked to perform the hand cooling during your breaks while the other group will go about their normal routine during their breaks and will not be allowed to use the hand cooling device

Study Timeline:



Baseline Data Collection:

Your child will be asked to provide a body height and body fat percentage measurement before your first day of data collection. Skin measurements will be taken at three sites (chest, abdomen & thigh) where a researcher will lightly "pinch" these sites in order to calculate their body composition. During your child's baseline they will also be oriented to the questionnaires and the scales that they will be asked to complete both before and after practice sessions. Your child will be asked to take a temperature pill that reads the temperature inside their body. They will be educated and instructed on how and when to take the temperature pill for the following day and give written instuctions to take home.

Data Collection Before Practice:

Prior to each practice we will confirm that the temperature pill your child took is working. Your child will then be asked to provide a small urine sample to measure hydration status and markers of muscle breakdown. This sample will not be used for any other reason and discarded immediately after it is analyzed. Body weight will then be taken while only wearing shorts. Next, your child will be given one of two GPS units to track your movement during practice. One unit fits inside a vest with a small GPS unit attached to the back between the shoulder blades or a small pouch that can be attached under the pads. The other unit is a GPS watch that will be worn on the wrist. Your child will also be asked to wear a heart rate strap in order to monitor their heart rate during practice. Both GPS units will collect their distance and speed as well as their heart rate during practice and store this information within the GPS device. If your child is wearing the watch, they will not be able to see the information on the watches other than the running time during practice. Next, your child will be asked to complete the Environmental Symptoms Questionnaire, pain, fatigue, recovery, sleep, thirst, thermal, and Delayed Onset Muscle Soreness scales. Your child then will get dressed for practice and head out onto the field.

Data Collection During Practice:

During practice, your child will participate as normal. When your child is given breaks for water or rest, players in the **cooling group only** will be asked to perform the hand cooling treatment during the break while players in the **non-cooling group** will do what they normally would do. At this time a researcher will also be taking the body temperatures **of both groups**. Your child will also be given a personal water bottle to drink from throughout practice so that we can measure the amount of fluid they drink during practice. We ask that your child drink only from your water bottle and avoid pouring the fluid over their head. We may also attempt to take measures of your child's thirst, thermal sensation, and rating of perceived exertion depending on the amount of time that they have for the scheduled break. There will be no interruptions to football practice for data collection during this study.

Data Collection Post Practice

Following practice, your child will be asked to complete the environmental symptoms questionnaire and muscle soreness scales, as well as the thirst, thermal, pain, fatigue, and recovery scales. Then, they will be asked to take off the GPS device and the heart rate strap. Your child will then be weighed and asked to provide a small urine sample. At the conclusion of each day they will be provided with another temperature pill to take that night before they go to sleep.

Your child will not know the results of the data pertaining to the study until a few months after the completion of the study.

In total, excluding time your child will already be spending in practice, the total anticipated time commitment for this study is estimated to be (30 minutes before practice on day 1 and day

4, and 15 minutes before and after every other practice). In total this amounts to approximately 2 hours over the 4 days. Data collection during the practice will change depending on the number of breaks given by your child's coaches. Data collection will occur in the athletic training facility and on the practice field each day.

Data Collection: Values represent when the variable will be collected and on which day

Variable	Collection Time	Baseline Testing	Day 1	Day 2	Day 3	Day 4
Height	1min	X				
Body Wt (Pre & post)	10 sec	X	X	X	X	X
Body Composition	2 min	X				
Urine Sample (Pre & post)	2 min		X	X	X	X
Environmental Temp/Wind Speed	Every 15 min		X	X	X	X
Body Temperature	During Each Break		X	X	X	X
Fluid Intake	During Each Break		X	X	X	X
Heart Rate	Continuous		X	X	X	X
Timex GPS	Continuous		X	X	X	X
Catapult GPS	Continuous		X	X	X	X
Perceptual Questionnaires						
Exercise and Football History	3 min	X				
Environmental Symptoms Questionnaire (Pre & Post)	30 sec		X	X	X	X
PAIN (Pre & Post)	10 sec		X	X	X	X
Muscle Soreness Scale (Pre & Post)	10 sec		X	X	X	X
Thirst (Pre, During, Post)	10 sec		X	X	X	X
Thermal (Pre, During, Post)	10 sec		X	X	X	X
Fatigue (Pre & Post)	10 sec		X	X	X	X
Quality Recovery (Pre)	10 sec		X	X	X	X
Rating of Perceived Exertion (During)	10 sec		X	X	X	X
Sleep Record	1 min		X	X	X	X

(PRE)						

Photo Release – During All Study Procedures

During the study your child may have their photograph taken should you choose. You will be offered a release form that allows University of Connecticut researchers to use your child's photo as part of scientific presentations or publications related to the study procedures. These photos help the researchers to accurately portray the methods of the study. Should you prefer not to sign the release form that will not change your child's ability to participate in this study. This is your own personal preference.

What are the risks or inconveniences of the study?

There is a risk associated with the temperature pills if certain health conditions exist, however we will screen "at-risk" individuals prior to enrollment and those who are considered to be at risk will not be allowed to participate. Those who are at risk include those who are suspected to have obstructive disease of the gastrointestinal tract, included but not limited to diverticulitis and inflammatory bowel disease, impaired gag reflex, previous gastrointestinal surgery, might undergo Nuclear Magnetic Resonance scanning, have hypomotility disorder of the gastrointestinal tract, or have a cardiac pacemaker or other electro medical device.

There are no known risks or discomforts associated with measurements of body weight, skin folds, heart rate, GPS, urine specific gravity, questionnaires or scales.

What are the benefits of the study?

Your child will receive his/her results of their practice sessions regarding the influence of hand cooling on their core temperature and performance during practice. Your child also will benefit from having information regarding their general fitness and health such as body fat percentage, sweat rate, and hydration status.

This study will increase knowledge of the effects of hand cooling on core body temperature and performance during high school football practices in the heat. This may improve athletic performance for athletes and help medical professionals prevent heat related injuries. The results of this study may influence and encourage further education of health care providers regarding the importance of hydration and cooling. Your child's participation in this study may benefit the general population, by allowing researchers, athletic trainers and coaches to determine the extent to which hand cooling effects core body temperature and performance.

Will my child receive payment for participation? Are there costs to participate?

You will not receive payment for participation in this study.

How will my child's information be protected?

The following procedures will be used to protect the confidentiality of the data collected from your child. All of your child's personal information collected as part of this study will remain confidential. Recorded information will remain in a locked cabinet in the Human Performance Laboratory and/or the Principle Investigators' office at the University of Connecticut. Your child will only be identified by an anonymous participant number on data sheets (ie Subject 01). There will only be one master list of these participant numbers that will be stored in a locked cabinet in the principal investigator's office. Information will be accessible only by the principle investigator and the student researchers. All participant information will be kept on file for seven years.

All electronic files (e.g., database, spreadsheet, etc.) containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by unauthorized users. Only the members of the research staff will have access to the passwords. Data that will be shared with others will be coded as described above to help protect your identity. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be identified in any publications or presentations. We will do our best to protect the confidentiality of the information we gather from your child but we cannot guarantee 100% confidentiality.

You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

What happens if my child is injured or sick because he/she took part in the study?

In the event your child becomes sick or injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If your child requires medical care for such sickness or injury, their care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed.

However, if you believe that your child's illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut or the State of Arkansas Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802 or the Office of Compliance at the University of Arkansas at 479-575-2208

Can my child stop being in the study and what are my and my child's rights?

Your child does not have to be in this study if you do not want him/her to participate. If you give permission for your child to be in the study, but later change your mind, you may withdraw your child at any time. There are no penalties or consequences of any kind if you decide that you do not want your child to participate. You will be notified of all significant new findings during the course of the study that may affect your child's willingness to continue. If necessary, your child may be withdrawn from the study at any time. Examples of withdrawal considerations are safety/medical concerns, missed appointments, non-adherence to procedures, disruptive behavior during study procedures, and/or adverse reactions.

Whom do I contact if I have questions about the study?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this study or if you have a research-related problem, you may contact the principal investigator, Douglas Casa, 860-420-9150 or Robert Huggins, 203-804-2316 or Brendon McDermott 479-575-4670. If you have any questions concerning your rights as a research participant, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802 or the University of Arkansas Institutional Review Board (IRB) at 479-575-2208.

Parental Permission Form for Participation in a Research Study

Principal Investigator(s): Douglas J. Casa, PhD, ATC and Brendon P. McDermott, PhD, ATC
Student Researcher: Robert Huggins, M.Ed, ATC
Study Title: The Effect of Intermittent Hand Cooling on Body Temperature and Performance During High School Football Practice in the Heat

Documentation of Permission:

I have read this form and decided that I will give permission for my child to participate in the study described above. Its general purposes, the particulars of my child's involvement and possible risks and inconveniences have been explained to my satisfaction. I understand that I can withdraw my child at any time. My signature also indicates that I have received a copy of this parental permission form. Please return this form to the child's coach or Athletic Trainer by (August 9th, 2013).

____________________	____________________	__________
Child Signature:	Print Name:	Date:

____________________	____________________	__________
Parent/Guardian Signature:	Print Name:	Date:

Relationship to Child (e.g. mother, father, guardian):
____

____________________	____________________	__________
Signature of Person Obtaining Consent	Print Name:	Date:

Appendix E.

Consent Form for Participation in a Research Study

University of Connecticut

Principal Investigator: Douglas J. Casa, PhD, ATC
Student Researcher: Robert Huggins, M.Ed, ATC
Study Title: The Effects of Hand-Cooling on Body Temperature, Power, Speed, Agility and Balance While Wearing Football Equipment in the Heat

Introduction

You are invited to participate in a research study to examine the effects of intermittent hand cooling on core body temperature and measures of performance while exercising in the heat in football equipment. Minimizing the rise in core body temperature via cooling during exercise in the heat has been shown to increase performance in measures of strength and endurance. However, past studies have not examined the use of an easy to use hand-cooling device on measures of performance while wearing equipment in the heat.

You are being asked to participate because you are a healthy male who exercises regularly (3 times per week or at least 6 hours per week).

This consent form will give you the information you will need to understand why this study is being done and why you are being invited to participate. It will also describe what you will need to do to participate and any known risks, inconveniences or discomforts that you may have while participating. We encourage you to take some time to think this over and to discuss it with your family, friends and doctor. We also encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form.

Why is this study being done?

The purpose of this investigation is to examine the effect of intermittent hand cooling (with and without hydration) on core body temperature, exercise performance, and physiological markers of stress and recovery during 3 bouts of 90 minutes of exercise (1 control exercise condition, 1 exercise condition with intermittent hand cooling while dehydrated, and 1 exercise condition with intermittent hand cooling hydrated) in a hot environment while wearing football equipment.
Research questions include:

1. What is the effect of hand cooling with and without fluid replacement during exercise breaks have on mitigating the rise of core body temperature while wearing football equipment?
2. What is the effect of hand cooling with and without fluid replacement during exercise breaks have on physiological markers of stress following one exercise bout and repeated exercise bouts while wearing football equipment?
3. What is the effect of hand cooling with and without fluid replacement during exercise breaks have on measures of exercise performance such as agility, speed, muscular power, and balance while wearing football equipment?

What are the study procedures? What will I be asked to do?

You may be included if you are a male between the ages of 18-35 and perform regular exercise either via weight training or aerobic exercise at a minimum of twice per week or greater than 3 hours per week. You will be screened via medical history questionnaire to ensure that you meet the following criteria: 1) no known chronic health problems that prohibit you from physical activity, 2) no previous history of exertional heat stroke within the past 3 years, 3) no known history of cardiovascular, metabolic or respiratory disease, 4) no known current musculoskeletal injury that limits physical activity.

If you agree to take part in this study, you will be asked to partake in 4 visits (one familiarization session, followed be 3 testing sessions) that will take place 1 day per week for 4 weeks of data collection. The 3 test session conditions will be conducted in random order and are described below.

One session you will receive a hand cooling treatment, another session you will receive no treatment, and lastly one session you will receive hand cooling treatment while maintaining baseline hydration status. Hand cooling consists of inserting your hand into a cooling device that circulates water and provides gentle pressure to the hand. The majority of data collection will occur before, during and after each testing session. During all research sessions, you will be asked to wear full football equipment, skin temperature sensors, a rectal probe to measure core temperature, and a heart rate monitor. You will be asked provide a urine sample so we can accurately assess your hydration level at the start of each testing session.

Familiarization:

During the familiarization session you will be fitted for the football equipment that you will be wearing during the research sessions. We will also educate and provide you instructions on self-insertion of the rectal probe, blood draw procedures, urine cup, and body mass procedures. You will be given the chance to familiarize yourself with the treadmill exercise for 30 minutes. During that time your sweat rate will be calculated for the fluid replacement trial. Next you will be given the chance to practice the exercise performance test that will be used during this study (e.g. vertical jump, agility board, treadmill sprint, and balance test). You will also be introduced to perceptual scales looking at your rating of perceived exertion, thirst, fatigue, and thermal sensation. You will then be asked to undergo a DEXA scan, performed by a trained radiologist to assess body fat percentage. The DEXA scan is a common measure where you will be asked to lie still on a bed for 10-15minutes while your body is scanned for fat and lean tissue content. Last, you will be educated on how to log a diet and fluid record because you will be asked to replicate that diet/fluid consumption on the day prior to each testing session.

Baseline Measures:

Prior to each testing session you will be asked to provide a small urine sample to measure hydration status and insert the rectal probe in the privacy of the restroom. Baseline measurement of body weight, attachment of the skin temperature sensors, and heart rate strap will be performed and then you will be asked to enter the heat chamber, which will be maintained at 98 degrees Fahrenheit at 30-40% relative humidity. After 10 minutes of equilibration to the heat. You will be asked to provide a small blood sample (14 mL) to measure the level of stress biomarkers. Next, you will be asked to complete the Environmental Symptoms Questionnaire (ESQ), perceived thirst, perceived thermal, and rating of perceived exertion scales (RPE), perceived thirst, and fatigue scales. Once all measurements have been taken you will be asked to put on your football equipment.

When all equipment is securely fastened you will perform baseline performance tests described below.

Performance Tests

Counter Movement Vertical Jump:

The performance assessment will require you to perform a counter movement vertical jump task. You will stand with two feet flat on the force plate, drop the arms, and then flex the hip, knee, and ankle before exploding upward at takeoff with the object of jumping as high as possible. The use of a drop-step technique will be encouraged. You will be allowed to step back with one foot then return the same foot to the initial starting position before jumping. The height will be determined by the force and the amount of time that the participant takes to return to the force plate. A force plate will be used to measure the vertical ground reaction force (VGRF) during the performance of the jump. The peak vertical ground reaction force will be normalized to body weight (N) for each participant (% body weight). Velocity and power will be calculated from the VGRF data using the impulse-momentum theorem. Force-time data will be used to calculated velocity and power for the entire duration of each jump. You will be asked to perform three trials of each task will be completed, but will be repeated if performed incorrectly.

Agility Test for Foot Speed and Reaction:

This portion of the performance assessment will require you to perform two agility tests using a feedback device connected to a reaction mat called the Quick Feet Board (The Quick Board LLC). The board consists of a rubber mat positioned on the ground with the sensor pads in five locations (upper right and left, lower right and left, and center). This mat is connected to a power cord and run to a control device that provides visual stimulus (five bright lights that correspond to the five foot pads) and feedback information about the results of the movement responses. The control pad also allows for the command of the types of drills. You will be asked to perform three repetitions of the Foot Speed (FS) drill, which involves a maximum number of foot touches during a 10-second interval separated by 90 seconds. You will then be asked to perform three repetitions of the REACT drill (3), which includes 10 foot touches separated by 60 seconds.

6 Second Sprint Speed Test:

This portion of the performance assessment will require you to perform three 6 second sprints on a non-motorized treadmill. You will be asked to start by walking for 30 sec then ramp up your speed until maximum speed is obtained over a period of 2 seconds and then continue that maximal effort for 6 seconds. Between each bout 2 minutes of active recovery will take place and the protocol will be repeated until three 6 second sprints have been obtained. You will be reminded to ramp down the speed while holding onto the bars sides of the treadmill. Top speed and distance covered will be recorded.

Balance Error Scoring System (BESS):

The BESS tests balance ability in two different stances on two surfaces on both legs. You will stand with yours eyes closed and hands on hips during all four tests. The two stances include: Single Leg Stance, and Tandem Stance. The Single Leg Stance requires you to stand on one foot with your opposite hip flexed 30 degrees and knee flexed 45 degrees. You will stand with the heel of one foot touching the toes of the other foot during the Tandem Stance. The two surfaces are a firm, flat surface and a foam pad.

You are instructed to assume the standard testing position and remain as still as possible for 20 seconds. You are scored based on the number of errors performed during the 20-second time period. Errors include: Lifting hands off the iliac crest, opening the eyes, stepping, stumbling, or falling, moving the hip into more than 30 degrees of flexion or abduction, lifting the forefoot or heel, or remaining out of the testing position for more than 5 seconds. If you cannot maintain the standard position for at least 5 seconds, the score for that trial is a 10 (the maximum score for each trial). You will perform one practice trial in each condition followed by 2 test trials in each condition.

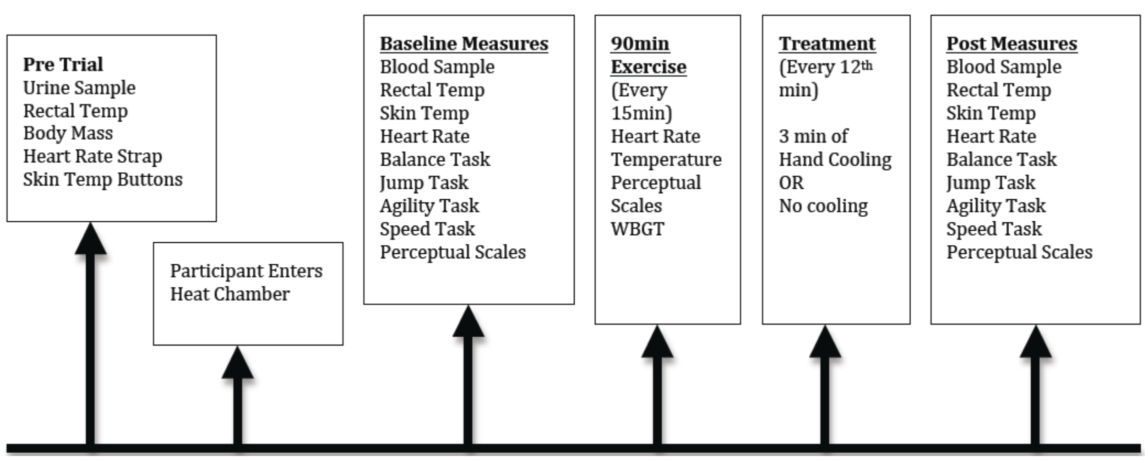
Exercise Session

After all baseline measurements are completed you will be asked to walk on a treadmill for 90 minutes at a speed of 3.5-4.5mph at a 5% incline. Your speed range will be determined in the familiarization session. During this time you will not receive fluid replacement unless you are in the hydration trial condition. At minute 12, 27, 42, 57, 72, and 87 you will be asked to step off of the treadmill and sit for 3 minutes while you get one of two treatments. You will receive one of the following:
1) no hand cooling 2) hand cooling or 3) hand cooling with fluid replacement during the trial. We will observe changes in core and skin temperature, as well as keep a record of changes in your ESQ, RPE, perceived thirst and perceived thermal sensations throughout the entire session.

Post-Test Data Collection

Following the exercise session you will be asked to perform the same performance tests that were completed in the beginning of the visit. You will also be asked to complete the psychological scales again (ESQ) as well as the thirst, thermal, rating of perceived exertion, fatigue, and recovery scales. You will then be asked to provide a small sample (14mL). Lastly, you will be weighed and sent to the restroom to remove the rectal thermometer and to provide a small urine sample.

Total time commitment for each session is about 11 hours (2 hours for familiarization and 3.0 hours for each testing session). In total you will provide 84mL of blood which is equivalent to 5.7 tablespoons of blood.



Study Procedures Timeline: (order of Trials 1, 2, and 3 will vary)

Variable	**Familiarization Session**	**Trial 1 (Hand Cooling with fluid loss)**	**Trial 2 (Hand Cooling without fluid)**	**Trial 3 (Control Trial)**

Pre & Post Body Mass	X	X	X	X
Height	X			
Heart Rate	X	X	X	X
Rectal Temperature	X	X	X	X
Skin Temperature	X	X	X	X
Pre and Post Urine Sample for (USG and Color)	X	X	X	X
DEXA Scan Test	X			
Sweat Rate Test	X			
Hand Cooling Device	X	X	X	
Pre and Post Vertical Jump	X	X	X	X
Pre and Post Agility Test	X	X	X	X
Pre and Post Treadmill Sprint	X	X	X	X
Pre and Post Balance Test	X	X	X	X
Pre and Post Blood Draw		X	X	X
ESQ Scale Every 15 min	X	X	X	X
Thermal Scale Every 15 min	X	X	X	X
Thirst Every 15 min	X	X	X	X
RPE Scale Every 15 min	X	X	X	X
Fatigue Every 15 min	X	X	X	X
Pain Every 15 min	X	X	X	X
WBGT Every 15 min	X	X	X	X

Photo Releases – During All Study Procedures

During the study you may have your photograph taken should you sign the release. You will be offered a release form that allows University of Connecticut researchers to use your photo as part of scientific presentations or publications related to the study procedures. These photos help the researchers to accurately portray the methods of the study. Should you prefer not to sign the release form that will not change your ability to participate in this study. Compensation for study participation will remain the same regardless of signing the release. This is your own personal preference.

What are the risks or inconveniences of the study?

The risks of participation in this study are as follows. It is possible that you will experience musculoskeletal injury, exercise-induced muscle cramps, or delayed onset muscle soreness. It is possible that you may strain a muscle, sprain a ligament or tendon, or incur a stress fracture in bone. Other possible risks include: (a) a fall during the treadmill walking or performance tests (b) a subcutaneous hematoma (i.e., bruise) may appear at the site of blood sampling; (c) although very unlikely, it is possible that a disturbance of heart rhythm will occur, (d) blood draw procedures may cause light-headedness or dizziness, (e) X-ray radiation from the body fat "DEXA" scan may cause abnormal changes in you bodies cells, (f) the risk of symptomatic exertional heatstoke is very low, due to the continuous physiological monitoring of rectal temperature and clinical signs and symptoms, (g) rectal thermometry may cause inconvienience due to any disscomfort you may have with insertion, removal, and movement with the device.

<u>Risk Mitigation:</u>

The following steps will be taken to limit the aforementioned risks.

1. Your known medical history forms will be reviewed for contraindications to vigorous exercise and a history of heat illness
2. You will be educated about the symptoms and signs of heat exhaustion and heatstroke, with instructions to stop exercise if these symptoms or signs develop
3. Your temperature and heart rate as well as clinical signs and symptoms will be monitored throughout the sessions.
4. At least two of the experimenters present at testing will have basic Red Cross CPR certification to provide you treatment if necessary.
5. A faculty investigator will be in Gampel Pavilion during all exercise sessions, performance testing, and blood draws.
6. Exercise testing and performance testing will be terminated if *one* of the following criteria is met: (a) signs and symptoms indicate that your health or safety is compromised; (b) if you verbally disclose that you choose to stop the training session. (c) your heart rate > 190 beats•min-1 for 5 consecutive minutes; (d) your rectal temperature > 40ºC; (e) you show signs and symptoms of heat exhaustion or heatstroke. (Note: it is very unlikely that any you will reach one of these safety limits, considering the ambient temperature and exercise protocol.) A certified athletic trainer, with expertice in the prevention, recognition, and treatment of exertional heat illness, will be on site during each exercise session.
7. A controlled, prescribed 10 min standing heat equilibration session will precede all testing sessions to allow you to equilibrate to the environment.
8. Musculoskeletal injury risks will be minimized by thorough instruction of the task to the you by one of the researchers as well as time for you to practice sprinting on a non-motorized treadmill, performing a vertical jump, performing agility drills, and balance testing in the familiarization sessions. Researchers will also be positioned on either side of the force platform while you performing the vertical jump, balance task, and treadmill sprint.
9. If a musculoskeletal injury occurs, you will have access to a certified Athletic Trainer for treatment between exercise sessions.
10. Only a trained and approved HPL blood draw researcher will draw blood via butterfly needle insertion, using aseptic technique and universal precautions. To avoid hematoma, you will be instructed to hold a sterile gauze pad on the vein from which blood is sampled.
11. Following laboratory testing sessions, you will be provided with water for rehydration.
12. If deemed necessary by a certified Athletic Trainer, cold water cooling will be available to decrease body temperature, in the locker room across the hallway from the Human Performance Laboratory.

13. The total volume of blood sampled during the entire study is 84 mL (6x 14mL draw sessions; is equivalent to about 5.7 tablespoons of blood. This represents ~ 1% of your total blood volume (5 L). For reference, a pint of blood which is commonly donated is equvalent to 473mL or 5.6 times the volume required for this investigation.
14. Any damage to your cells from the x-ray will be minimal. The body continuously repairs any small changes and the amount of radiation is very low in this study. The total exposure for the whole body scan is approximately 125 times less than the average radiation from a standard chest X-ray.

<u>Other Safeguards That Will Be Employed</u>

1. One or more laboratory personnel trained in CPR and the use of an automatic external defibrillator (a device that electrically stimulates the heart to restore rhythm in people who are experiencing cardiac arrest) will be present during all exercise sessions. In the unlikely event of an emergency during exercise (e.g., incidence of 1 in 250,000 for arrhythmia), 911 will be called and physician Jeffrey Anderson will be alerted. Dr. Anderson will be informed of all testing schedules, prior to performance tests.
2. Emergency protocols for handling a cardiovascular incident and/or hyperthermia are in place and all personnel (including certified Athletic Trainers among the graduate student investigators) are trained in rapid response, including the use of EMT services (telephone 911). A Kinesiology faculty member will be present in the Kinesiology department for all testing sessions and run training workouts.
3. You will be instructed to inform investigators regarding any new (or revised) medications that they take, any medical/dental treatment, or any illness experienced during this investigation. You also should report any adverse physical or mental reactions that may be related to this study. You will be instructed not to donate blood (i.e., at a Red Cross Blood Drive) during the 8 weeks before this study, or during this study.

What are the benefits of the study?

You will receive results of your tests regarding the influence of hand cooling on your body's ability to regulate core temperature as well as performance testing measures. You also will benefit from having information regarding your general fitness and health such as body fat percentage, sweat rate, and hydration status.

This study will increase knowledge of the effects of hand cooling with and without hydration on core body temperature and performance during exercise in the heat while wearing full football equipment. This may improve athletic performance for athletes and help medical professionals prevent heat related injuries. The results of this study may influence and encourage further education of health care providers regarding the importance of hydration and cooling. Your participation in this study may benefit the general population, by allowing researchers, athletic trainers and coaches to determine the extent to which hand cooling effects core body temperature and performance.

Will I receive payment for participation? Are there costs to participate?

You will be compensated monetarily for your time and effort. You will receive up to $250. If you complete only part of the study you will be compensated for that portion based on the outline below.

Phase Completed	
Baseline measurement	$45
Testing Session 1	$45
Testing Session 2	$45
Testing Session 3	$45
Completion Bonus	$70
Total Possible Compensation	**$250**

You will not be reimbursed for any parking.

How will my personal information be protected?

All participant information collected as part of this study will remain confidential. Recorded information will remain in a locked cabinet in the Human Performance Laboratory and/or the Principal Investigators' office. When information is entered into computer databases (that is password protected), the information will not include participant identifiable information. Participants will only be identified by an anonymous participant number on data sheets. There will only be one master list of these participant numbers that will be stored in a locked cabinet in the principal investigator's office. Information will be accessible only by the principle investigator and the student researchers. All participant information will be kept on file for seven years.

All electronic files (e.g., database, spreadsheet, etc.) containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by unauthorized users. Only the members of the research staff will have access to the passwords. Data that will be shared with others will be coded as described above to help protect your identity. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be identified in any publications or presentations.

You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

What happens if I am injured or sick because I took part in the study?

In the event you become sick or injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If you require medical care for such sickness or injury, your care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed.

However, if you believe that your illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802."

Can I stop being in the study and what are my rights?

You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate. You will be notified of all significant new findings during the course of the study that may affect your willingness to continue. If necessary, you may be withdrawn from the study at any time. Examples of withdrawal considerations are safety/medical concerns, missed appointments, non-adherence to procedures, disruptive behavior during study procedures, and/or adverse reactions.

Whom do I contact if I have questions about the study?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this study or if you have a research-related problem, you may contact the principal investigator, Douglas Casa, 860-420-9150 or the Robert Huggins, 203-804-2316. If you have any questions concerning your rights as a research participant, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Documentation of Consent:

I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks and inconveniences have been explained to my satisfaction. I understand that I can withdraw at any time. My signature also indicates that I have received a copy of this consent form.

__________________ ___________________________

Participant Signature: Print Name: Date:

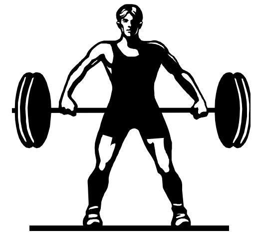
Relationship (only if not participant):____________________________

__________________ ___________________________

Signature of Person Obtaining Consent Print Name: Date:

Appendix F. Laboratory Study Recruitment Flyer

Volunteers Wanted For A Research Study



“The Effects of Hand Cooling on Body Temperature, Power, Speed, Agility and Balance While Wearing Football Equipment in the Heat”

Are you between the ages of 18-35?
Do you enjoy exercise?
Do you want to know your personal measures of power, speed, agility, and balance?

The University of Connecticut is now enrolling for a new study with the goal of determining the performance benefits of a simple hand cooling device during exercise in the heat.

For participation you will receive;
1) Knowledge of your power, speed, agility, and balance
2) Knowledge of your personal sweat rate and percent body fat
4) Up to $250 compensation for only 4 visits

We are recruiting 14 healthy males who lift weights or exercise 3 times per week or at least 6 hours per week.

For more information contact;

Robert Huggins
University of Connecticut
(203) 804-2316
robert.huggins@uconn.edu

Study conducted under the direction of Douglas Casa, Ph.D.
This study was approved by the UConn IRB, Protocol # H13-053

Appendix G:

SLEEP RECORD

Subject #______________

Rob Huggins
Phone- (860)486-6520
Email-
Robert.Huggins@uconn.edu

Rob Huggins
Phone- (860)486-6520
Email-
Robert.Huggins@uconn.edu

Rob Huggins
Phone- (860)486-6520
Email-
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Email-
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Date of Record (if recording this in the AM, list the date of the previous day):_____________

Time went to sleep:__________

Time awoken:___________

Total amount of sleep (record to the half hour, e.g., 7.5hrs):______________

How would you rate your sleep quality? (Circle)

Poor Fair Good Great

How rested do you feel overall? (Circle)

Minimally-rested Fairly-rested Rested Well-rested Optimally-rested

Did you take any naps yesterday?

No_________

Yes________

Number of hours (record to the half hour):_____________Time nap occurred:____________

Appendix H.

ENVIRONMENTAL SYMPTOMS QUESTIONAIRE

Subject #: _____________ Date:_____________________

How Do You Feel Questionnaire

1. Place an X in the box to explain HOW YOU HAVE BEEN FEELING TODAY.
2. PLEASE ANSWER EVERY ITEM.
3. If you did not have the symptom, say NOT AT ALL.

Symptoms	Not At All	A Little	Somewhat	Moderate	A Lot	Extreme
I feel lightheaded						
I have a headache						
I feel dizzy						
I feel thirsty						
I feel weak						
I feel grumpy						
It is hard to breathe						
I will play at my best						
I have a muscle cramp						
I feel tired						
I feel sick to my stomach (nauseous)						
I feel hot						
I have trouble concentrating						
I have "goose bumps" or chills						

4. SOURCE: Modified from Kobrick and Sampson (1979) and Sampson and Kobrick (1980).

PAIN INTENSITY SCALE

0	**NO PAIN AT ALL**
½	**VERY FAINT PAIN (just noticeable)**
1	**WEAK PAIN**
2	**MILD PAIN**
3	**MODERATE PAIN**
4	**SOMEWHAT STRONG PAIN**
5	**STRONG PAIN**
6	
7	**VERY STRONG PAIN**
8	
9	
10	**EXTREMELY INTENSE PAIN (almost unbearable)**
•	**UNBEARABLE PAIN**

Subject:__________________ **Date**: _______________

EVALUATION OF MUSCLE SORENESS

"On the horizontal line below please put a small vertical line across this horizontal line that best describes the pain you feel. A vertical mark on the extreme LEFT side of the line would indicate that you are experiencing 'no soreness'; a vertical mark on the extreme RIGHT side of the line would indicate that you are experiencing 'unbearable pain'.

If your degree of pain is somewhere in between these two extremes, please mark it at the place that most accurately describes your current pain level.

Please mark the line to indicate the pain that you are experiencing now."

Indicate your muscle soreness on this scale:

No Soreness **Unbearable Pain**

____ 10 mm

Thermal Scale

0	**Unbearably Cold**
1	**Very Cold**
2	**Cold**
3	**Cool**
4	**Comfortable**
5	**Warm**
6	**Hot**
7	**Very Hot**
8	**Unbearably Hot**

Thirst Scale

1 Not Thirsty At ALL

2

3 A Little Thirsty

4

5 Moderately Thirsty

6

7 Very Thirsty

8

9 Very, Very Thirsty

Total Quality Recovery

6	No recovery at all
7	Extremely poor recovery
8	
9	Very poor recovery
10	
11	Poor recovery
12	
13	Reasonable recovery
14	
15	Good recovery
16	
17	Very good recovery
18	
19	Extremely good recovery
20	Maximum recovery

<u>Fatigue Scale</u>

INDICATE YOUR LEVEL OF OVERALL FATIGUE <u>RIGHT NOW</u>

0 No Fatigue At All

1 Very Small Amount of Fatigue

2 Small Amount of Fatigue

3 Moderately Fatigued

4 Somewhat Fatigued

5 Fatigued

6

7 Very Fatigued

8

9 Extremely Fatigued

10 Completely Fatigued

Borg Rating of Perceived Exertion (RPE) Scale & Instructions

While doing physical activity, we want you to rate your perception of exertion. This feeling should reflect how heavy and strenuous the exercise feels to you, combining all sensations and feelings of physical stress, effort, and fatigue. Do not concern yourself with any one factor such as leg pain or shortness of breath, but try to focus on your total feeling of exertion.

Look at the rating scale below while you are engaging in an activity; it ranges from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion." Choose the number from below that best describes your level of exertion. This will give you a good idea of the intensity level of your activity, and you can use this information to speed up or slow down your movements to reach your desired range.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Your own feeling of effort and exertion is important, not how it compares to other people's. Look at the scales and the expressions and then give a number.

6 No exertion at all	**13 Somewhat hard**
7 Extremely light	**14**
8	**15 Hard (heavy)**
9 Very light	**16**
10	**17 Very hard**
11 Light	**18**
12	**19 Extremely hard**
	20 Maximal exertion

9 corresponds to "very light" exercise. For a healthy person, it is like walking slowly at his or her own pace for some minutes
13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.

17 "very hard" is very strenuous. A healthy person can still go on, but he or she really has to push him- or herself. It feels very heavy, and the person is very tired.

19 on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.

A larger version of the actual size of the scale used is on the next page.

RATING OF PERCEIVED EXERTION SCALE

6	
7	Very, Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, Very Hard
20	

BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit

Instruction Manual

Preparing Human Inflammatory Cytokines Standards

Purpose of this procedure

The Human Inflammatory Cytokines Standards are lyophilized and should be reconstituted and serially diluted immediately before mixing with the Capture Beads and the PE Detection Reagent.

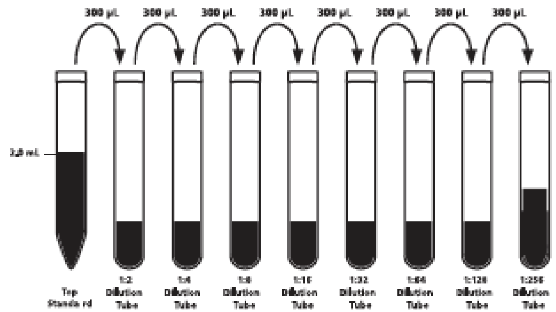
You must prepare fresh standards to run with each experiment. Do not store or reuse reconstituted or diluted standards.

Procedure

To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized Human Inflammatory Cytokine Standards. Transfer the standard spheres to a 15-mL polypropylene tube. Label the tube "Top Standard."
2. Reconstitute the standards with 2 mL of Assay Diluent.
    a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.
    b. Gently mix the reconstituted protein by pipette only. Do not vortex or mix vigorously.
3. Label eight 12 × 75-mm tubes and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.
4. Pipette 300 µL of Assay Diluent in each of the 12 × 75-mm tubes.
5. Perform a serial dilution:
    a. Transfer 300 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipette only. Do not vortex.

b. Continue making serial dilutions by transferring 300 µL from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube.



6. Prepare one 12 × 75-mm tube containing only Assay Diluent to serve as the 0 pg/mL negative control.

Concentration of standards

See the Performing the Human Inflammatory Cytokine Assay (page 24) for a listing of the concentrations (pg/mL of all six recombinant proteins in each standard.

Next step

Proceed to Mixing Human Inflammatory Cytokine Capture Beads (page 20).

Mixing Human Inflammatory Cytokine Capture Beads

Purpose of this procedure

The Capture Beads are bottled individually (A1–A6). You must pool all six bead reagents immediately before using them in the assay.

Mixing the beads

To mix the Capture Beads:

1. Determine the number of assay tubes (including standards and controls) that are required for the experiment (for example, 8 unknowns + 9 standard dilutions + 1 negative control = 18 assay tubes).
2. Vigorously vortex each Capture Bead suspension for 3 to 5 seconds before mixing.

   Note: The antibody-conjugated beads will settle out of suspension over time. Vortex the vial immediately before taking a bead-suspension aliquot.

3. Add a 10-µL aliquot of each Capture Bead, for each assay tube to be analyzed, into a single tube labeled "Mixed Capture Beads" (eg, 10 µL of IL-8 Capture Beads × 18 assay tubes = 180 µL of IL-8 Capture Beads required).
4. Vortex the bead mixture thoroughly.

Resuspending the beads

If you are using serum or plasma samples, you must perform this procedure to reduce the chances of false-positive results due to serum or plasma proteins. This procedure is optional for all other sample types.

To resuspend the Capture Beads in Serum Enhancement Buffer:

1. Centrifuge the mixed Capture Beads at 200g for 5 minutes.
2. Carefully aspirate and discard the supernatant.

3. Resuspend the mixed Capture Beads pellet in Serum Enhancement Buffer (equal to the volume removed in step 2) and vortex thoroughly.
4. Incubate the mixed Capture Beads for 30 minutes at room temperature, protected from light.

Next step

The mixed Capture Beads are now ready to be transferred to the assay tubes. Discard excess mixed Capture Beads. Do not store after mixing.

To begin the assay, proceed to Performing the Human Inflammatory Cytokine Assay (page 24). If you need to dilute samples having a high-protein concentration, proceed to Diluting samples (page 22).

Diluting samples

Purpose of this procedure

The standard curve for each protein covers a defined set of concentrations from 20 to 5,000 pg/mL. It might be necessary to dilute test samples to ensure that their mean fluorescence values fall within the range of the generated standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given protein. This procedure is not required for all samples.

Procedure

To dilute samples with known high-cytokine concentration:

1. Dilute the sample by the desired dilution factor (for example, 1:2, 1:10, or 1:100) using the appropriate volume of Assay Diluent.

   Optimal recovery from serum samples typically requires a 1:4 dilution.
2. Mix sample dilutions thoroughly.

Next step

Perform instrument setup using the Cytometer Setup Beads. For details, go to bdbiosciences.com/cbasetup and select the appropriate flow cytometer under CBA Kits: Instrument Setup.

Or, if you wish to begin staining your samples for the assay, proceed to Performing the Human Inflammatory Cytokine Assay (page 24), and you can perform instrument setup during the 3-hour staining incubation.

To perform the assay:

1. Vortex the mixed Capture Beads and add 50 µL to all assay tubes.

2. Add 50 µL of the Human Inflammatory Cytokine Standard dilutions to the control tubes as listed in the following table.

Tube label	Concentration (pg/mL)	Standard dilution
1	0 (negative control)	no standard dilution (Assay Diluent only)
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1,250	1:4
9	2,500	1:2
10	5,000	Top Standard

3. Add 50 µL of each unknown sample to the appropriately labeled sample tubes.

4. Incubate the assay tubes for 1.5 hours at room temperature, protected from light.

   Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation, or during the incubation in step 8.

5. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.

6. Carefully and consistently aspirate and discard the supernatant, leaving approximately 100 µL of liquid in each assay tube.

7. Add 50 µL of the Human Inflammatory Cytokine PE Detection Reagent to all assay tubes. Gently agitate the tubes to resuspend the pellet.

8. Incubate the assay tubes for 1.5 hours at room temperature, protected from light.

9. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.

10. Carefully aspirate and discard the supernatant from each assay tube.

11. Add 300 µL of Wash Buffer to each assay tube to resuspend the bead pellet.

To perform the assay:

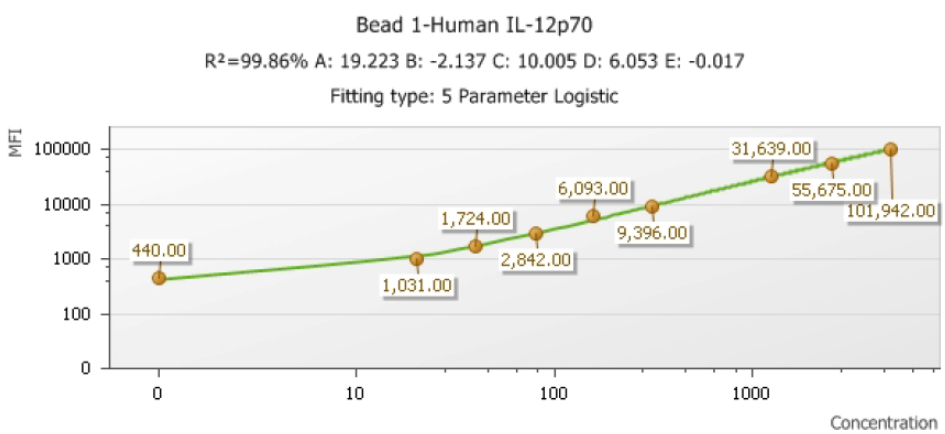
1. Wet the plate by adding 100 µL of wash buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2 to 10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
5. Add 50 µL of each of the following to the wells in the filter plate:
   - Capture Beads (vortex before adding)
   - Standard or sample (add standards from the lowest concentration to the highest, followed by samples)
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for 1.5 hours at room temperature on a non-absorbent, dry surface.

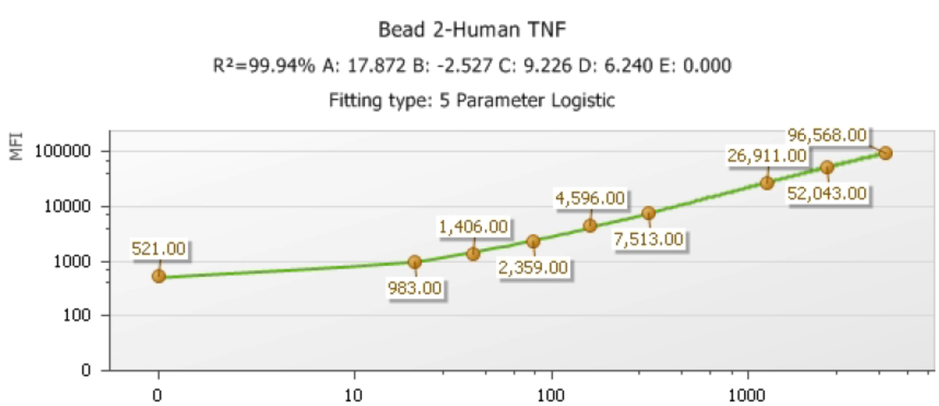
   Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
8. Remove the cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2 to 10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
11. Add 200 µL of wash buffer to each well. Cover the plate and shake for 2 minutes at 1,100 rpm.
12. Repeat step 8 through step 10.
13. Add 100 µL of assay diluent to each well.
14. Add 50 µL of Human Inflammatory Cytokine PE Detection Reagent to each well.
15. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
16. Incubate the plate for 1.5 hours at room temperature on a non-absorbent, dry surface.

    Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
17. Repeat step 8 through step 10.
18. Add 120 µL of wash buffer to each well to resuspend the beads.
19. Shake the plate for 2 minutes at 1,100 rpm before you begin sample acquisition.

Plex Components

		Analyte		
Name	Lot Number	Name	Model	2nd Reporter
Bead 1		Human IL-12p70	Quantitative	No
Bead 2		Human TNF	Quantitative	No
Bead 3		Human IL-10	Quantitative	No
Bead 4		Human IL-6	Quantitative	No
Bead 5		Human IL-1β	Quantitative	No
Bead 6		Human IL-8	Quantitative	No

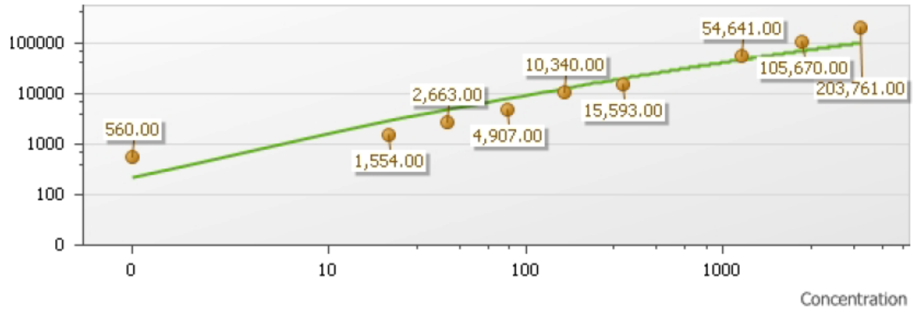




Bead 3-Human IL-10

R²=91.82% A: 16.762 B: -6.211 C: 39.964 D: -6.071 E: -40.033

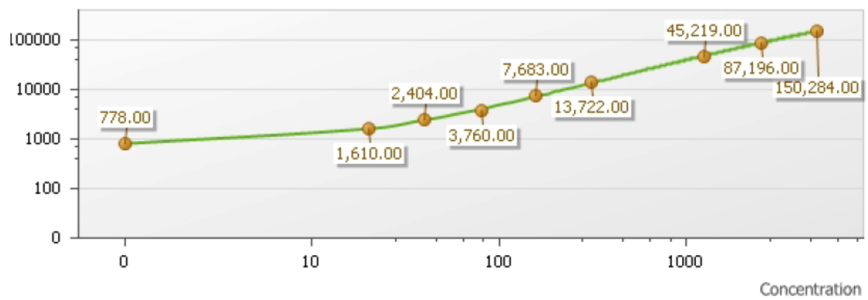
Fitting type: 5 Parameter Logistic



Bead 4-Human IL-6

R²=99.95% A: 16.583 B: -2.618 C: 8.156 D: 6.659 E: -0.012

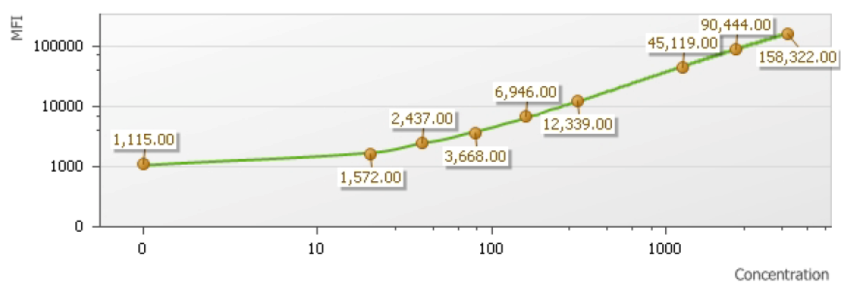
Fitting type: 5 Parameter Logistic



Bead 5-Human IL-1β

R²=99.94% A: 16.466 B: -3.058 C: 8.317 D: 6.973 E: -0.122

Fitting type: 5 Parameter Logistic



Bead 6-Human IL-8

R²=99.87% A: 17.555 B: -2.622 C: 8.771 D: 7.356 E: -0.092

Fitting type: 5 Parameter Logistic